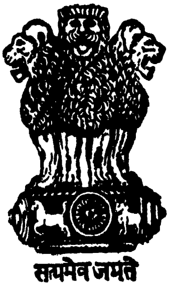

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ANNALS OF TROPICAL MEDICINE
AND PARASITOLOGY

THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR WARRINGTON YORKE, M.D., F.R.S.
PROFESSOR D. B. BLACKLOCK, M.D.
PROFESSOR W. S. PATTON, M.B.
PROFESSOR R. M. GORDON, M.D.

VOLUME XXVII

(April 10, 1933, to December 20, 1933)

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CONTENTS

No. 1. April 10, 1933

PAGE

ADAMS, A. R. D.
Report on a Collection of Nematodes from the Federated Malay States .. 1

GIBBINS, E. G.
The Domestic *Anopheles* Mosquitoes of Uganda 15

DAVEY, T. H.; and GORDON, R. M.
The Estimation of the Density of Infective Anophelines as a Method of Calculating the Relative Risk of Inoculation with Malaria from Different Species or in Different Localities 27

GORDON, R. M.; and DAVEY, T. H.
A Further Note on the Increase of *P. malariae* in Freetown, Sierra Leone .. 53

THEILER, MAX.
A Yellow Fever Protection Test in Mice by Intracerebral Injection .. 57

MACFIE, J. W. S.
A New Species of *Culicoides* from Palestine 79

DE MEILLON, BOTHA.
On *Anopheles funestus* and its Allies in the Transvaal 83

DUKE, H. LYNDHURST.
Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. I.—A Review of Existing Knowledge on this Subject, with some General Observations 99

DUKE, H. LYNDHURST.
Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. II.—On the Transmitting Power of Different Races of *Glossina* 119

DUKE, H. LYNDHURST.
Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. III.—*Glossina morsitans versus Glossina palpalis* as a Transmitter of the Polymorphic Group of Trypanosomes 123

CHATTERJI, R. C.
On a New Nematode, *Parapharyngodon maplestoni* gen. nov., sp. nov., from a Burmese Lizard 131

PATTON, W. S.
Studies on the Higher Diptera of Medical and Veterinary Importance. A Revision of the Genera of the Tribe Muscini, Subfamily Muscinae, based on a Comparative Study of the Male Terminalia. I.—The Genus *Musca* Linnaeus 135

YORKE, WARRINGTON; MURGATROYD, FREDERICK; and HAWKING, FRANK.
Studies in Chemotherapy. X.—Further Observations on the Transmissibility of Tryparsamide-Resistance by Glossina 157

MISCELLANEA 179

CONTENTS

No. 2. July 7, 1933

FAIRBAIRN, H. PAGE

The Action of Human Serum *in Vitro* on Sixty-four Recently Isolated Strains of *T. rhodesiense* 185

HUDSON, ELLIS H.; and YOUNG, AGNES L.

Hookworm Disease in the Arab of the Middle Euphrates 207

NIESCHULZ, OTTO.

Some Remarks about the Rôle of True Blood-Sucking *Musca* Species as Transmitters of Diseases 213

DUKE, H. LYNDHURST.

A Study of the Behaviour of *T. rhodesiense* Recently Isolated from Man, in Antelope and other African Game Animals. Part I 215

LANE, CLAYTON.

The Taxies of Infective Hookworm Larvae 237

FAIRBAIRN, H.

Experimental Infection of Man with a Strain of *Trypanosoma rhodesiense* Apparently Susceptible to Normal Human Serum *in Vitro* 251

EVANS, A. M.

Anopheles demeilloni sp. n., a New Name for *Anopheles transvaalensis* of Authors 265

EVANS, A. M.; and DE MEILLON, BOTHA.

Notes on *Anopheles demeilloni* (*transvaalensis*) and *Anopheles garnhami* in South Africa, with Descriptions of New Varieties of these Species 271

STEPHENS, J. W. W.

The Distribution of Blackwater Fever in Central America, South America and the West Indies 283

ADAMS, A. R. D.

A Record of an Investigation into the Action of Sera on the Trypanosomes Pathogenic to Man 309

PATTON, W. S.

Studies on the Higher Diptera of Medical and Veterinary Importance. A Revision of the Species of the Genus *Musca*, based on a Comparative Study of the Male Terminalia. II.—A Practical Guide to the Palaearctic Species 327

YORKE, WARRINGTON; MURGATROYD, FREDERICK; and HAWKING, FRANK.

The Relation of Polymorphic Trypanosomes, Developing in the Gut of Glossina, to the Peritrophic Membrane 347

MISCELLANEA 355

CONTENTS

No. 3. October 21, 1933

PAGE

LESTER, H. M. O.

The Characteristics of some Nigerian Strains of the Polymorphic Trypanosomes 361

PATTON, W. S.

Studies on the Higher Diptera of Medical and Veterinary Importance. A Revision of the Species of the Genus *Musca*, based on a Comparative Study of the Male Terminalia. II.—A Practical Guide to the Palaearctic Species. (*con.*) 397

DUKE, H. LYNDHURST.

Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. IV.—On the Spontaneous Disappearance of Flagellates from an Infected Glossina.. 431

DUKE, H. LYNDHURST.

Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. V.—On the Effects of Temperature 437

DUKE, H. LYNDHURST.

Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. VI.—On the Duration of the Biological Cycle in Glossina 451

SHUTE, P. G.

A Simple Method of Obtaining Eggs of Mosquitoes 469

ADAMS, A. R. D.; and WEBB, LEWIS.

Two Further Cases of Human Infestation with *Bertiella studeri* (Blanchard, 1891) Stiles and Hassall, 1902, with some Observations on the Probable Synonymy of the Specimens Previously Recorded from Man 471

PATTON, W. S.

A New Haematophagous Species of *Musca* from Malaya 477

MISCELLANEA 481

CONTENTS

No. 4. December 20, 1933

PAGE

MER, G.

Observations on the Development of *Plasmodium malariae* Lav. in *Anopheles elutus* Edw.: Experimental Transmission of Quartan Malaria to Man from the Mosquito 483

GIBBINS, E. G.; and LOEWENTHAL, L. J. A.

Cutaneous Onchocerciasis in a *Simulium damnosum*-infested region of Uganda 489

PEASTON, H.

Preliminary Note on a Focus of *S. mansoni* Infection in Sierra Leone.. .. 497

PATTON, W. S.

Studies on the Higher Diptera of Medical and Veterinary Importance. A Revision of the Tribe Muscini, Subfamily Muscinae, based on a Comparative Study of the Male Terminalia. II.—The Genus *Stomoxys* Geoffroy (sens. lat.) 501

CUSHING, EMORY C.; and PATTON, W. S.

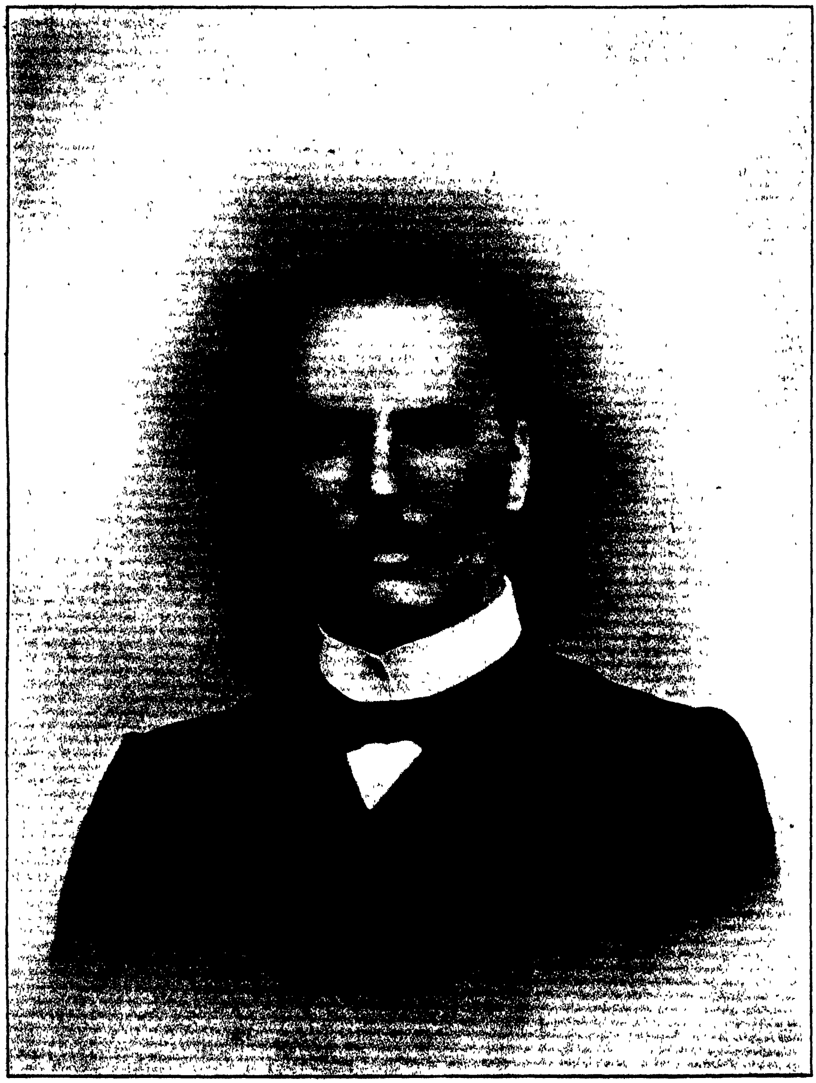
Studies on the Higher Diptera of Medical and Veterinary Importance. *Cochliomyia americana* sp. nov., the Screw-Worm Fly of the New World .. 539

WALLACE, J. M.

The Polymorphic Trypanosomes of Damba Island, Victoria Nyanza. III.—Their Reactions in Laboratory Animals 553

DUKE, H. LYNDHURST.

Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. VII.—*T. rhodesiense versus T. gambiense*: a Comparison of their Power to Develop Cyclically in Glossina 569



Ronald Ross

IN MEMORIAM

The Editors regret to announce the death on September 16th, 1932, of SIR RONALD ROSS, K.C.B., K.C.M.G.

In 1899, soon after his epoch-making discovery of the mosquito transmission of malaria, Major Ross was appointed Lecturer in the newly-established Liverpool School of Tropical Medicine. In 1903, the Alfred Jones Chair of Tropical Medicine was founded and Ross was elected the first Professor. He occupied this Chair until the end of 1912 when he went to London: for the next five years he still retained a position on the Staff of the School as Professor of Tropical Sanitation. The photograph reproduced was taken in 1908 while Ross was in Mauritius studying malaria on the 20th Expedition of the School.

Volume XXVII April 10, 1933 No. 1

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

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This fellowship was founded in 1929 to commemorate the late Dr. Richard Caton, one of the original members of the governing body of the School, and for many years its Vice-Chairman.

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(1) Two courses for the Diploma in Tropical Medicine, commencing on the 2nd October, 1933, and the 3rd January, 1934. The D.T.M. examinations are held in December, March and June.

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REPORT ON A COLLECTION OF NEMATODES FROM THE FEDERATED MALAY STATES

BY

A. R. D. ADAMS

(*Received for publication* 12 *July*, 1932)

This collection was made by Mr. G. B. Purvis, F.R.C.V.S., of the Federated Malay States Veterinary Department, and submitted by him for identification. It is now in the collection of the Liverpool School of Tropical Medicine. The major portion of the collection came from Malay, though a considerable number of specimens was obtained in this country and elsewhere. In all, some 300 tubes of material were examined and the genera determined; these are enumerated below, together with the hosts from which they came and their places of origin. Owing to the mass of material, it has not been possible to determine all the species present up to now, but this has been done at least for the Strongyloidea. In recording the names of the hosts and the position of the parasites in them, the collector's memoranda in the bottles are given. Certain new genera and species are recorded after the enumeration of the parasites identified.

Superfamily **STRONGYLOIDEA**

Family TRICHOSTRONGYLIDAE

Subfamily TRICHOSTRONGYLINAE

Genera and species—	Found in -
Haemonchus contortus	21 tubes of material from cattle, sheep and goats in Taiping, Raub and Nebong Tibal.
Ostertagia circumcincta	4 tubes, 1 from a buffalo in Kamunting, and the remainder from sheep in Taiping, and 1 in Kenya Colony.

	Found in—
Molineus torulosus	1 tube from a squirrel monkey in Edinburgh Zoological Gardens.
Cooperia punctata	4 tubes, 3 from cattle in Raub and Maxwell's Hill, and 2 from sheep in Taiping and Seremban.
C. pectinata	1 tube from a calf in Kenya Colony.
C. curticei	1 tube from a sheep in Berwick, N.B.
Trichostrongylus extenuatus	3 tubes, 1 each from a sheep, a goat and a bison in Taiping, Raub and the Edinburgh Zoological Gardens respectively.
T. colubriformis	9 tubes, 6 from goats in Taiping and Raub, and 3 from sheep in Seremban, Alor Star and Kenya Colony respectively.
T. rugatus	2 tubes from sheep in Taiping.
T. axei	1 tube from a horse in Taiping.
T. probolurus	1 tube from a sheep in Kenya Colony.
Libyostrongylus douglassii	1 tube from an ostrich in Kenya Colony.
Nematodirus filicollis	1 tube from a sheep, locality unknown.
Mecistocirrus digitatus	2 tubes from cattle in Kamunting and Taiping.

Family ANCYLOSTOMIDAE

Subfamily NECATORINAE

Genera and species—	Found in—
Necator americanus	1 tube of material from man in Taiping.
Gaigeria pachyscelis	6 tubes, 5 from goats in Taiping and Alor Star, and 1 from a sheep in Taiping.
Bunostomum phlebotomum	2 tubes, 1 from an ox in Raub and the other from a calf in Nebong Tibal.
B. trigonocephalum	1 tube from a sheep in Taiping.

Subfamily ANCYLOSTOMINAE

Genera and species—	Found in—
Ancylostoma braziliense	23 tubes of material from dogs and cats in Taiping, Raub, Seremban and Dinding's.
A. caninum	21 tubes from dogs in Taiping and Seremban.
A. duodenale	1 tube from man in Taiping.
A. malayanum	1 tube from a rat in Seremban. This would appear to be the first occasion on which an ancylostome has been recorded from the rat, and it is interesting to note that the parasite broadly conforms to the description of *Ancylostoma malayanum* given by Lane (1916). It would thus appear undesirable to accord these specimens new specific rank solely on account of their host, and I feel that they should be regarded as examples of *A. malayanum* from a previously unrecorded host. The specimens consist of 2 males and 2 females, and are in excellent condition. They are numbered 1657 in the nematode collection of the Liverpool School of Tropical Medicine.

Family STRONGYLIDAE

Subfamily OESOPHAGOSTOMINAE

Genera and species—	Found in—
Oesophagostomum radiatum	5 tubes of material from cattle in Taiping, Kedah and Maxwell's Hill.
O. columbianum	8 tubes, 6 from goats in Taiping and 2 from sheep in Taiping and Kenya Colony respectively.
O. venulosum	3 tubes from goats in Taiping.
O. asperum	1 tube from a goat in Taiping.
Chabertia ovina	3 tubes from a sheep in Taiping.

Subfamily TRICHONEMINAE

Genera and species—	Found in—
Gyalocephalus capitatus	1 tube of material from a horse in Taiping.
Trichonema spp.	12 tubes from mules, ponies and horses in Taiping, and 1, consisting of a single male specimen, from a fox in Niddrie, Scotland.

Subfamily STRONGYLINAE

Genera and species—	Found in—
Triodontophorus minor	3 tubes of material from horses and mules in Taiping.
T. brevicauda	2 tubes from horses in Taiping.
T. tenuicollis	2 tubes from ponies in Taiping and Ayton.
T. serratus	2 tubes from ponies in Taiping and Ayton.
Globocephalus urosubulatus	2 tubes from pigs in Kedah and Raub.
Strongylus vulgaris	10 tubes from horses, ponies and mules in Taiping and Ayton.
S. edentatus	4 tubes from ponies and horses in Taiping.
S. equinus	2 tubes from horses in Taiping.

Subfamily STEPHANURINAE

Genus and species—	Found in—
Stephanurus dentatus	3 tubes of material from pigs in Taiping and Ipoh.

Family SYNGAMIDAE

Genus and species—	Found in—
Syngamus trachea	3 tubes of material, 2 from fowls in Taiping, and 1 from a goose in Edinburgh, Scotland.

Family METASTRONGYLIDAE

Subfamily METASTRONGYLINAE

Genera and species—	Found in—
Metastrongylus salmi	8 tubes of material from pigs in Seremban and Taiping.
Dictyocaulus filaria	1 tube from a sheep in Africa.

Superfamily **OXYUROIDEA**

Family OXYURIDAE

Subfamily SYPHACIINAE

Genus and species—	Found in—
Syphacia obvelata	2 tubes of material from rats in Taiping and Pabang.

Subfamily OXYURINAE

Genus and species—	Found in—
Oxyuris equi	3 tubes of material from mules and ponies in Taiping.

Family HETERAKIDAE

Genus and species—	Found in—
Heterakis spp.	20 tubes of material from fowls and a turkey in Taiping and elsewhere ; also 1 from a knot in Edinburgh Zoological Gardens and 1 from a rat in Taiping. In addition, there is a tube of material from a cat in Taiping which contains a number of specimens of this genus ; these have possibly been ingested by the cat, and cannot therefore be regarded as true parasites of the animal.

Family SUBULURIDAE

Subfamily SUBULURINAE

Genus–	Found in—
Subulura sp.	1 tube of material from a knot in Edinburgh Zoological Gardens.

Superfamily **SPIRUROIDEA**

Family ACUARIIDAE

Subfamily ACUARIINAE

Genus—	Found in—
Acuaria spp.	5 tubes of material from fowls in Taiping.

Family GNATHOSTOMIDAE

Subfamily GNATHOSTOMINAE

Genus and species—	Found in—
Gnathostoma spinigerum	2 tubes of material from cats in Taiping.

Family PHYSALOPTERIDAE

Subfamily PHYSALOPTERINAE

Genus and species—	Found in—
Chlamydonema praeputiale	4 tubes of material from cats in Taiping and Seremban.

Family SPIRURIDAE

Subfamily GONGYLONEMINAE

Genus and species—	Found in—
Gongylonema ingluvicola	2 tubes of material from fowls in Raub and Taiping.

Subfamily SPIRURINAE

Genera and species—	Found in—
Spirocerca sanguinolenta	1 tube of material from a dog in Seremban.
Cyrnea sp.	1 tube of material from a knot in Edinburgh Zoological Gardens.
Habronema spp.	12 tubes from horses, ponies and mules in Taiping.

Subfamily ARDUENNINAE

Genus—	Found in—
Physocephalus sp.	1 tube of material from a dog in Taiping. The material contains a single damaged female worm which appears to belong to this genus.

Family THELAZIIDAE

Genera—	Found in—
Oxyspirura spp.	7 tubes of material from fowls in Taiping and Seremban.
Thelazia spp.	6 tubes from cattle in Seremban and Ipoh.

Superfamily **TRICHUROIDEA**

Family TRICHURIDAE

Subfamily TRICHURINAE

Genera—	Found in—
Trichuris spp.	6 tubes of material from cattle, sheep and goats in Taiping; 1 from a monkey, *Macaca irus*, in Raub; 1 from a coypu in Edinburgh Zoological Gardens; and 1 from man in Taiping.

Subfamily CAPILLARIINAE	Found in—
Capillaria spp.	4 tubes from fowls and turkeys in Taiping, and a sample from a knot in Edinburgh Zoological Gardens.

Superfamily **ASCAROIDEA**

Family ASCARIDAE

Subfamily ASCARINAE

Genera and species—	Found in—
Ascaris lumbricoides	1 tube of material from a pig in Taiping.
Toxocara spp.	7 tubes from dogs in Taiping and Seremban; 1 from a fox in Niddrie, Scotland; and another, consisting of 3 specimens, from a long-eared owl in Edinburgh Zoological Gardens.

Subfamily ASCARIDIINAE

Genus—	Found in—
Ascaridia spp.	5 tubes of material from fowls and a turkey in Taiping and Kuala Kangsar.

Superfamily **FILARIOIDEA**

Family FILARIIDAE

Subfamily SETARIINAE

Genera and species—	Found in—
Setaria hornbyi	2 tubes of material from an oribi and a reedbuck in Kenya Colony, and a sample from an ox in Seremban. This species of *Setaria* does not seem to have been found in Asia before; the specimens are in an excellent state of preservation and present apparently all the characters of this species.
S. labiato-papillosa	6 tubes from cattle and buffaloes in Taiping and Seremban.
S. digitata	1 sample from a bison in Edinburgh Zoological Gardens.

Subfamily ONCHOCERCINAE

Genera and species—	Found in—
Elaeophora poeli	3 tubes of material from cattle and a buffalo in Seremban and Taiping.
Onchocerca sp.	1 tube from an ox in Kedah.

Subfamily FILARIINAE

Genera and species—	Found in—
Dirofilaria immitis	3 tubes of material from dogs in Seremban and Ipoh.

Genus *CYCLODONTOSTOMUM* gen. nov.

C. PURVISI sp. nov.

Material collected by Mr. G. B. Purvis, F.R.C.V.S., of the Federated Malay States.

Source. Large intestine of a rat, from Raub, Federated Malay States, on 3.2.30.

Type specimens and cotypes in the collection of the Liverpool School of Tropical Medicine, Number 1656.

The collection consists of 9 female and 7 male small filiform nematodes, with a shallow, chitinous, subglobular buccal capsule, the oral margin of which bears a number of teeth arranged symmetrically around it and directed into the buccal cavity. Transverse cuticular striation is present throughout the length of the worms. The mouth is directed obliquely dorsally and consists of a prominent, circular, ring-shaped, chitinous buccal capsule, about 100μ in diameter and 65μ in depth; this capsule is continuous with the oesophagus, the latter being somewhat dilated at its anterior end. Six-head papillae are present, and consist of dorsal, ventral and lateral pairs, and there is a prominent cephalic groove. A shallow dorsal oesophageal groove is present. Round the inner margin of the buccal capsule are arranged symmetrically 8 pairs of strongly curved teeth, which project into the oral cavity; they measure from 35μ to 48μ, those situated ventrally being the longest, and those dorsally, the smallest. In the depths of the buccal capsule and on its dorsal surface are 2 small triangular lancets. The oesophagus is about 550μ in length and is dumbell-shaped, but has no valvular apparatus at its posterior extremity. The nerve-ring is situated about the middle of the oesophagus. Cervical papillae could not be seen.

The males measure 8 to 9 mms. in length and about 300μ in diameter. The bursa is symmetrical and has a large dorsal lobe. The dorsal ray is bifurcate in its distal third, each bifurcation ending in two digits. The externo-dorsal ray is long, and arises from a common trunk with the dorsal. The postero-lateral and medio-lateral rays are parallel and close together, whilst the strong externo-lateral ray arises from a common trunk with the laterals. The ventral ray is cleft. The spicules are subequal and measure approximately 650μ. There is no gubernaculum.

The females measure 1·2 to 1·3 cms. in length, and roughly 400μ in diameter. The vulva is about 400μ in front of the anus, and the two parallel uteri run forward together. The tail of the female is conical. The eggs are of the usual thin-shelled strongylate type and are oval, measuring about 53μ by 34μ.

I propose the generic name of *Cyclodontostomum* for this worm, and have much pleasure in dedicating the species to the collector, Mr. Purvis.

The morphology of the worm obviously relegates it to the super-family **STRONGYLOIDEA,** family ANCYLOSTOMIDAE and subfamily ANCYLOSTOMINAE, although the family character of the oral aperture being guarded by ventral cutting organs would appear now to require some modification, as the cutting organs in this genus are arranged symmetrically around the mouth on the dorsal and ventral surfaces equally. It would thus appear that the character of the family ANCYLOSTOMIDAE should be 'oral aperture guarded by cutting organs, i.e. teeth, or chitinous plates,' without specifying the position of these cutting organs.

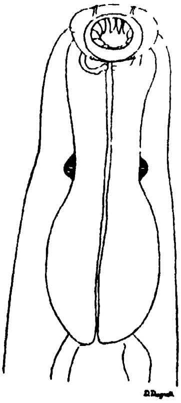

FIG. 1. *Cyclodontostomum purvisi* n.gen. and sp. Dorsal view of anterior extremity. (× 70.)

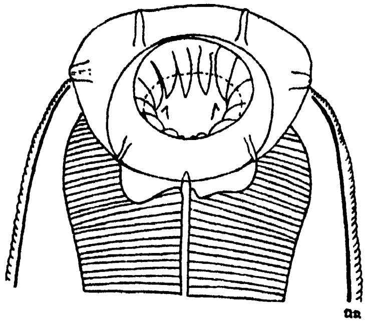

FIG. 2. *Cyclodontostomum purvisi* n.gen. and sp. Dorsal view of head. (× 180.)

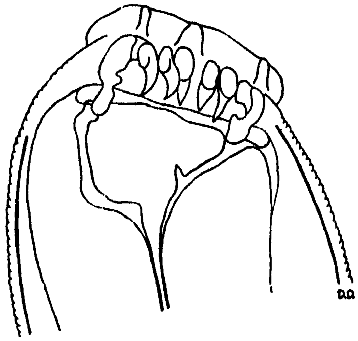

FIG. 3. *Cyclodontostomum purvisi* n.gen. and sp. Lateral view of head. (× 180.)

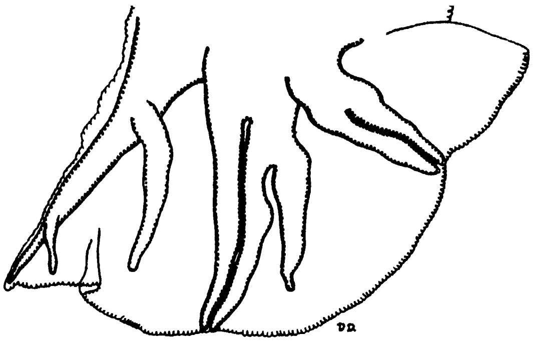

FIG. 4. *Cyclodontostomum purvisi* n.gen. and sp. Lateral view of bursa. (× 180.)

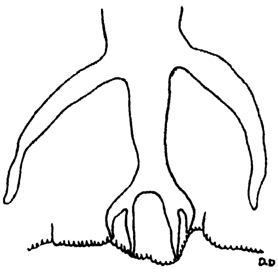

FIG. 5. *Cyclodontostomum purvisi* n.gen. and sp. Dorsal view of bursa. (× 180.)

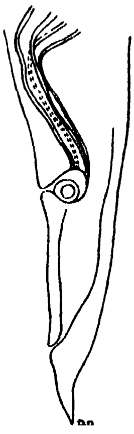

FIG. 6. *Cyclodontostomum purvisi* n.gen. and sp. Lateral view of posterior extremity of female. (× 40.)

Genus *LABIATOFILARIA* gen. nov.

L. TRINGAE sp. nov.

Material collected by Mr. G. B. Purvis, F.R.C.V.S.

Source. Peritoneal cavity of a 'knot' in Edinburgh Zoological Gardens.

Type specimens in the collection of the Liverpool School of Tropical Medicine, Number 1675.

The material consists of a male and a female worm ; these are in a good state of preservation, except that the spicules of the male are both broken off. They are relatively large, stout worms, the female being about three times the length of the male. The cuticle is smooth and not bossed, and is finely transversely striated throughout the length of the worms. The mouth is simple and unprovided with any chitinous structures ; the buccal capsule is rudimentary, and no vestibule is present. Two prominent lateral lips bind the mouth, and each of these lips is deeply cleft, thus giving the appearance of four prominent, symmetrical, sublateral lips. The head is furnished with lateral and submedian papillae, and small lateral cervical papillae are present. The oesophagus is divided into two parts, a relatively short anterior muscular portion and a longer posterior glandular portion. Around the anterior muscular portion lies the nerve-ring. The tails of both the male and female worms are abruptly rounded, the anus being subterminal in each case.

MALE. About 17 mm. in length and 640μ in breadth. The tail is bluntly rounded, and there are a number of preanal and postanal sessile papillae. There are 2 spicules, but these unfortunately have been broken off short. No gubernaculum is present, and there are no caudal alae.

FEMALE. About 60 mm. in length and 1 to 1·5 mm. in breadth. The vulva is situated in the posterior oesophageal region, 800μ from the anterior extremity of the worm. The vagina is very muscular and from it 2 parallel uteri arise. The posterior extremity of the worm is bluntly rounded and the anus is subterminal ; both in front of and behind the anus are a number of sessile papillae.

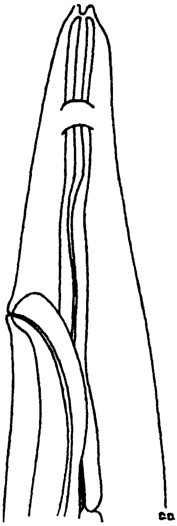

FIG. 7. *Labiatofilaria tringae* n.gen. and sp. Lateral view of anterior extremity. (× 40.)

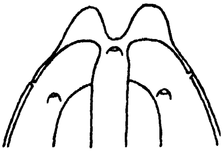

FIG. 8. *Labiatofilaria tringae* n. gen. and sp. Lateral view of head. (× 180.)

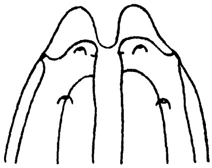

FIG. 9. *Labiatofilaria tringae* n.gen. and sp. Dorso-ventral view of head. (× 180.)

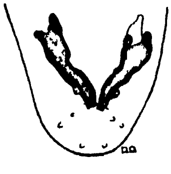

FIG. 10. *Labiatofilaria tringae* n. gen. and sp. Ventral view of posterior extremity of male. (× 70.)

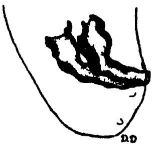

FIG. 11. *Labiatofilaria tringae* n.gen. and sp. Lateral view of posterior extremity of male. (× 70.)

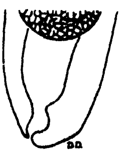

FIG. 12. *Labiatofilaria tringae* n.gen. and sp. Lateral view of posterior extremity of female. (× 40.)

This worm appears obviously to belong to the superfamily **FILARIOIDEA,** and can be referred to the family FILARIIDAE. In that the mouth is simple, the cuticle is finely transversely striated and the vulva lies in the oesophageal region, it would seem to belong either to the subfamily APROCTINAE or the subfamily FILARIINAE. Unfortunately, as the spicules are damaged, it is impossible to say whether they were equal and similar, or dissimilar, but examination of the remaining portions suggests that they were similar; if this is indeed the case, the worm can be referred to the subfamily APROCTINAE. The prominent lips are a striking feature on first examination and appear to differentiate it from any of the other genera in these two families, and I am unable to find any description of a similar worm elsewhere, with the possible exception of *Tetracheilonema quadrilabiatum* (Diesing, 1861). This worm, however, is so insufficiently described that it is difficult to assign the parasite just described to this genus. I, therefore, propose the generic name of *Labiatofilaria* for its reception, and the specific name *tringae* from the host from which it was obtained.

REFERENCE

LANE, CLAYTON (1916). The genus *Ancylostoma* in India and Ceylon. *Ind. Jl. Med. Res.*, **4**, 74.

CORRIGENDUM

'A study of the house-haunting Culicidae occuring in Freetown, Sierra Leone,' by R. M. Gordon, E. P. Hicks, T. H. Davey and M. Watson, Vol. XXVI, No. 3, October, 1932.

Page 344, line 33, '——— and PHILIP, C. B. (1931)' should read 'DAVIS, G. E., and PHILIP, C. B. (1931).'

THE DOMESTIC *ANOPHELES* MOSQUITOES OF UGANDA

BY

E. G. GIBBINS

(*Received for publication* 7 *November*, 1932)

CONTENTS

		PAGE
I.	PRESENCE AND PREVALENCE OF MALES IN DWELLINGS	17
II.	INFLUENCE OF DOMESTIC ANIMALS	21
III.	INFLUENCE OF SMOKE	23
IV.	INFLUENCE OF VEGETATION	24
V.	SUMMARY	25

The present paper is derived from detailed records and notes kept during an infectivity survey of the domestic *Anopheles* of Uganda, which was the subject of a recent paper (Gibbins, 1932), and is intended as a contribution to the study of the house-frequenting habits of *Anopheles* mosquitoes.

Our knowledge of the habits of malaria-carrying *Anopheles* is extremely meagre. Clayton Lane (1931), in his critical summary of literature in regard to housing and malaria, shows clearly how little is known of this important subject. Our ignorance is partly due to the fact that it is seldom realised how much more informative from the point of view of malaria an adult survey of human dwellings may be, as opposed to a larval survey of breeding places. Johnson (1919) found that a totally false idea is obtained from a larval survey of a station, as the common larvae obtained may not be the common domestic mosquito. Ingram and De Meillon (1929) note that adults of *Anopheles costalis* Theo. (*gambiae* Giles) and *Anopheles funestus* Giles were found in places where, had reliance been placed on failure to find the larvae, these malaria carriers would have been reported as absent. In Uganda, two malaria-carrying species, *Anopheles funestus* and *Anopheles moucheti* Evans, which were rarely found as larvae in certain townships, were the predominant species in human dwellings.

Throughout the inquiry, which was spread over approximately four years and was conducted in four widely separated and physically different regions of the country, a detailed record was kept of the prevalence and infection of *Anopheles* in individual dwellings. Both at the commencement and subsequently, the various habitations which were subjected to bi-weekly searches for mosquitoes were personally visited at regular intervals. These inspections served the dual purpose of checking the work of the native collectors and noting any change in the original condition of the dwelling. The investigation was not without its misfortunes. Many of the selected native huts fell down, some were burnt, while others were deserted for various periods and rendered useless for the purpose of a comparative study.

The selected observation dwellings were searched twice weekly during the early morning and forenoon by the same native collectors. The mosquitoes were captured singly in test tubes of the size 6 ins. by ¾ in., excepting in the case of *Anopheles mauritianus*, when wider tubes, 6 ins. by 1 in., were used in order to avoid damage to their long hind legs. By inserting a small plug of cotton wool after each insect, and carefully pushing it down so as to imprison it in a small space, 5 or 6 mosquitoes from the same dwelling were confined in a single tube for transportation to the laboratory. This method of collection, suggested by James (1920), proved quite successful; the fragile insects arrived at the laboratory in good condition and enabled accurate determinations to be made of closely allied species. As the huts of the indigenous population were very dark inside, it was necessary to examine them with the aid of an electric torch.

Most of the specimens of *Anopheles costalis* and *Anopheles funestus* captured in native huts were taken on the upper portion of the walls and on the lower parts of the roof, in the darkest places of the dwelling. They were inclined to prefer the region of the junction of the wall and roof. Cobwebs were particularly favoured as resting-places. Few were found near the ground, excepting in the vicinity of beds which had been recently slept in. There was a tendency for males to congregate and rest within small areas; but the writer did not find, as Garnham (1929) did in the case of *Anopheles funestus* at Kisumu in Kenya, that the males preferred to rest near the ground and the females near the roof. Single isolated specimens of *Anopheles mauritianus* var. *ziemanni* (Grünb.)

Edw. were obtained on the high parts of walls, but the majority of examples were captured near the ground. In huts where this species was present in appreciable numbers, and in particular in those in which goats had been tethered for the night, this habit of resting near the ground was very noticeable. Objects such as tins and bottles standing on the ground were favoured resting-places.

I. PRESENCE AND PREVALENCE OF MALES IN DWELLINGS

Christophers, Sinton and Covell (1928) suggest that the presence or absence of males among catches of adult mosquitoes may be taken as an indication of whether breeding places exist at the time. The observations carried out in Uganda show clearly that it is only in the case of two species, *Anopheles costalis* and *Anopheles funestus*, that this factor can be taken into account. It will be seen from Table I that males of the other four malaria-carrying species occurring in the country were seldom found in human dwellings. Of 5,687 specimens of *Anopheles moucheti* Evans, 4, and out of 1,031 *theileri* var. *hancocki* Edw., 2 were males, while in the case of

TABLE I

Town	Collections in Human Dwellings															
	A. costalis		*A. funestus*		**A. theileri*		*A. moucheti*		*A. marshalli*		*A. pharoensis*		†*A. mauritianus*		*A. transvaalensis*	
	M	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
KAMPALA ...	818	8,295	614	7,115	2	1,029	—	—	—	—	—	—	—	—	—	—
JINJA	264	1,651	979	10,019	—	—	4	5,683	—	—	—	198	—	291	—	—
FORT PORTAL ...	—	11	38	557	—	—	—	—	—	997	—	—	—	—	1	452
TOTAL ...	1,082	9,957	1,631	17,691	2	1,029	4	5,683	—	997	—	198	—	291	1	452

* *A. theileri* var. *hancocki*. † *A. mauritianus* var. *ziemanni*.

Anopheles pharoensis Theo. and *Anopheles marshalli* Theo., of which 198 and 997 females respectively were captured, not a single male was identified. The collections of two other species of house-frequenting *Anopheles*, not found to be concerned in the transmission of malaria in Uganda, were also conspicuous for the absence of males. In the case of one species, *Anopheles transvaalensis* Carter, 1 male was obtained out of 453 captured, while in the instance of *Anopheles mauritianus* var. *ziemanni*, out of 291 specimens, no males were found.

Lamborn (1925) found that a relative abundance of males of *Anopheles costalis* and *Anopheles funestus* in native huts coincided with the increase of females. That this was not the case in Kampala is shown in the following Table (Table II), giving the monthly collections of both sexes for a period of 11 months. A relationship does not appear to exist between males and females of *Anopheles costalis* and *Anopheles funestus* habitually frequenting dwellings of the indigenous population.

TABLE II

Month	*Anopbeles costalıs*			*Anopheles funestus*		
	Females	Males		Females	Males	
		No.	%		No.	%
June...	542	64	10·6	372	18	4·6
July	525	60	10·3	857	118	12·1
August	500	74	12·9	767	85	10
September	393	54	12	584	66	10·1
October	784	106	12	823	77	7·4
November	1,518	165	9·2	640	82	11·2
December	728	61	7·8	646	33	5
January	798	54	6·3	583	29	4·7
February	657	64	8·9	575	39	6·3
March	727	69	8·7	536	44	7·6
April	502	47	8·5	550	22	3·8

With regard to the opinion often expressed that the prevalence of males in human dwellings is associated with their close proximity to breeding places, some evidence is tabulated below (Table III). The statement, which covers a period of 11 months, gives the total number of adult specimens collected from an equal number of native huts situated in six different localities in the neighbourhood of Kampala, and shows the proportion of males to females. The huts in localities C, D and E were in the midst of breeding places, those of the second being the more extensive ; while, in the case of localities A, B and F, the dwellings occupied positions on the slopes of hills a short distance away. A study of the data presented will show that the prevalence of males of the species *costalis* and *funestus* in native huts is independent of their distance from breeding places.

TABLE III

Locality	*Anopheles costalis*			*Anopheles funestus*			Remarks
	Males	Females	Approx. proportion	Males	Females	Approx. proportion	
A	114	962	1 : 8	8	64	1 : 8	Huts a short distance from breeding places.
B	100	1,530	1 : 15	7	140	1 . 20	"
C	32	426	1 . 13	3	69	1 : 23	Huts in the midst of breeding places.
D	322	3,290	1 : 10	434	5,388	1 . 12	"
E	159	1,211	1 : 8	143	1,195	1 : 8	"
F	91	876	1 : 9	19	259	1 : 14	Huts a short distance from breeding places.
Total ...	818	8,295	1 : 10	614	7,115	1 : 11	

In Uganda, the prevalence of males of *Anopheles costalis* and *Anopheles funestus* appears to be largely a question of light. It will be seen from Table IV that the bright dwellings of Europeans at Jinja, during 4 months' observation, yielded a much greater proportion of males than the dark native huts situated in a locality very much nearer breeding grounds.

TABLE IV

Month	European houses				Native huts			
	A. costalis		*A. funestus*		*A. costalis*		*A. funestus*	
	Males	Females	Males	Females	Males	Females	Males	Females
June	5	1	33	19	23	135	120	959
July	3	7	56	87	18	119	331	1,696
August	3	1	26	45	6	55	125	1,465
September	1	1	4	31	19	63	82	1,388
Total	12	10	119	182	66	372	658	5,508

The collections made in European houses to ascertain the common domestic species in three other townships in the Protectorate support this observation. The details of these collections, which were kindly placed at my disposal by Mr. G. L. R. Hancock, Assistant Entomologist, are shown in Table V. Except in one instance, that of *Anopheles funestus* at Lira, where no males were captured, a comparatively high figure for this sex is recorded.

TABLE V

Town	European houses			
	Anopheles costalis		*Anopheles funestus*	
	Males	Females	Males	Females
Soroti	20	37	67	114
Lira	65	58	...	10
Tororo	47	68	5	7
Total	132	163	72	131

II. INFLUENCE OF DOMESTIC ANIMALS

Symes (1930), at Taveta in Kenya, found a high human malaria parasite rate in dwellings infested with *Anopheles costalis* and *Anopheles funestus* which were occupied by both man and cattle, indicating that, in spite of easy accessibility to cattle, man was the favoured source of food. In the same district, Symes (1932) carried out precipitin tests on the stomach contents of 165 specimens of *Anopheles funestus* caught in dwellings shared by man and cattle, and found that only 6 examples contained the blood of the latter. The writer's observations on the habits of these two species in Uganda tend to support these findings. While in the case of *Anopheles mauritianus* var. *ziemanni*, huts in which both humans and domestic animals lived proved particularly attractive, the presence of animals did not appear to influence either prevalence or infectivity in the case of *Anopheles costalis* and *Anopheles funestus*, the most important malaria-carrying species in the country.

Table VI shows the prevalence and infection of *Anopheles costalis* and *Anopheles funestus* in two pairs of native dwellings, situated in the midst of extensive breeding grounds near Kampala. Of the first pair, goats and chickens were kept in hut D3 throughout the year's observation, while in the other hut domestic animals were absent. The two huts differed as follows:

Hut D2	Hut D3
3 men and 2 women	6 men, 2 women and 3 children
3 rooms	4 rooms
2 raised beds	3 raised beds
Domestic animals absent.	Goats and chickens kept.

They were both wattle and daub structures, thatched with grass and covered by a roof of petrol tins which had been opened out and laid on in the form of tiles. The rooms were small compartments, partitioned to a height of 5 to 6 feet and open to the roof, and with no inside doors. The second pair of huts, D10 and D11, were occupied by three women and one child, and one man and one woman, respectively. The inhabitants of both huts owned goats and chickens, but, whereas the owners of D10 housed them in a separate building, the occupants of D11 slept in the same hut as the animals. The dwellings were similar in every respect; they were wattle and daub, thatched with grass, and contained 4 rooms and 1 raised bed.

TABLE VI

Hut	*Anopheles costalis*				*Anopheles funestus*			
	Number caught		Number of females per room	Percentage infected	Number caught		Number of females per room	Percentage infected
	Males	Females			Males	Females		
D2	48	514	171	17·5	73	931	310	15·6
D3	66	774	193	16·3	74	853	213	18
D10	14	131	33	17·7	23	263	66	18·2
D11	16	128	32	18·2	22	269	67	17·9

It will be seen from Table VI that no appreciable difference in prevalence or infectivity exists between those huts in which both domestic animals and humans resided and those where man was the only occupant. If *Anopheles costalis* and *Anopheles funestus* favoured goats and chickens as a source of food, one would expect to find a higher infection rate and a relatively smaller number of these two species present in huts occupied by man alone. That the majority of *Anopheles* were captured in the inner rooms used by the human inhabitants and not in the outer room occupied by goats and chickens is additional evidence of a preference for human blood.

In the case of *Anopheles mauritianus* var. *ziemanni*, there is no doubt that the presence of domestic animals plays an important rôle in the house-frequenting habit of this non-malaria carrying species. Table VII shows the prevalence of *Anopheles* in 24 native huts situated in two localities in the neighbourhood of Jinja for a period of 4 months. An occasional goat was present in several huts, but only in one hut in each locality (B1 and E9) was a herd housed in the same dwelling as the human inhabitants. The figures are significant, and show clearly that *Anopheles mauritianus* prefers the blood of goats to that of man. It is also worthy of note that most of the specimens of this species when captured in these two dwellings were taken near the ground.

TABLE VII

Situation	Number of females caught				
	A. mauritianus var. *ziemanni*	*A. costalis*	*A. funestus*	*A. pharoensis*	*A. marshalli*
Hut B1	80	61	38	13	...
Average per hut for 11 other huts in Locality B	3	70	110	7	0·5
Hut E9	49	1	2	...	...
Average per hut for 11 other huts in Locality E	0·5	1·7	1·6	. .	0·3

III. INFLUENCE OF SMOKE

Garnham (1929), working at Kisumu in Kenya, observed that native dwellings with fires within contained few or no anophelines. Symes (1930), on the other hand, found no lack of *Anopheles* in native huts where the atmosphere was heavily charged with smoke and the roof inside black with carbon. In such huts at Isiolo and Taveta maximum catches were made. De Meillon (1930) records the presence of *Anopheles funestus* in smoke-filled native huts. The writer's observations at Kampala in Uganda show that the presence of smoke due to cooking fires within a native hut does not inhibit *Anopheles costalis* and *Anopheles funestus*.

Table VIII is a summary of one year's catches in 42 native huts distributed about 6 different localities in the neighbourhood of the town. The collections represent *Anopheles* from an equal number of huts in which it was customary to light fires and do the cooking inside, and of those where cooking was usually carried out in another hut situated nearby. The huts varied much in size and were provided with no means of ventilation other than a small doorway. As far as possible, dwellings with a similar number of rooms are equally divided between each group. All the huts under consideration had a separate apartment for sleeping. In cases where the hut consisted of a single room, the bed was partitioned or curtained off. It is therefore apparent that mosquitoes were able to find a place less impregnated with smoke than the rest of the hut.

From Table VIII it is clear that, while the number of *Anopheles costalis* and *Anopheles funestus* captured in huts containing smoke is higher than those without, there is little difference in their relative infectivity.

TABLE VIII

Condition of huts	*Anopheles costalis*			*Anopheles funestus*		
	Number caught		Percentage infected	Number caught		Percentage infected
	Males	Females		Males	Females	
21 native huts with smoke ...	284	2,687	18·1	196	2,181	16·3
21 native huts without smoke .	195	1,786	18·7	108	1,262	15·9

IV. INFLUENCE OF VEGETATION

Two huts, structurally similar, in which conditions remained much the same throughout the year's investigation, show strikingly how vegetation may influence mosquito density in native huts. They were situated on the slope of a hill near Kampala, some 500 yards apart, at almost equal distances from the same anopheline breeding grounds and in a locality where comparatively few *Anopheles* mosquitoes were caught.

Both dwellings were wattle and daub, thatched with elephant grass and partitioned into three small windowless rooms. A small door let into the east wall, kept open except at night and in the absence of the owner, led to a central apartment with a dark, badly ventilated room on either side. In each case cooking was done in another hut erected for this purpose nearby. In hut A1 resided an elderly man and woman, who kept their home and immediate vicinity clean, and followed an active life, cultivating an extensive area around it with sweet potatoes. The occupants of hut A2 were a man and two women, who paid little attention to their home, seldom cleaning the hut and allowing dense foliage of plantain trees and elephant grass to encircle its very walls.

The bi-weekly searches over a period of one year yielded 18 *costalis*, 2 *funestus* and 3 culicines in hut A1, and 217 *costalis*, 25 *funestus*, 17 *theileri* var. *hancocki* and 91 culicines in hut A2. It is significant from these figures that hut A2 was considerably more attractive both to the anopheline and to the culicine mosquito.

V. SUMMARY

1. The inference to be drawn from the presence and prevalence of males of *Anopheles* in dwellings is discussed. In the case of the species habitually frequenting human habitations in Uganda, prevalence of males does appear to be of much significance as indicating proximity or otherwise of breeding places or intensity of breeding. Evidence is presented which shows that in European houses males occur much more frequently than in native huts, and it is suggested that light may be a factor of importance.

2. The most dangerous malaria-carrying species of *Anopheles* in Uganda, *costalis* and *funestus*, show a preference for man, whereas *Anopheles mauritianus* var. *ziemanni*, which occurred in appreciable numbers in human dwellings in one township, but was not incriminated in the natural transmission of malaria, was definitely attracted to native huts by the presence of goats.

3. Smoke of the amount usually produced by wood fires when cooking is carried out within a native hut does not appear to influence infestation by *Anopheles costalis* and *Anopheles funestus*.

4. Dwellings surrounded by dense vegetation were found to be particularly attractive to *Anopheles* mosquitoes in Kampala.

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THE ESTIMATION OF THE DENSITY OF INFECTIVE ANOPHELINES AS A METHOD OF CALCULATING THE RELATIVE RISK OF INOCULATION WITH MALARIA FROM DIFFERENT SPECIES OR IN DIFFERENT LOCALITIES*

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CONTENTS

		PAGE
I.	Introduction ...	27
II.	Previous Methods of Calculating the Anopheline Infective Density ...	29
III.	Definition of the Proposed Formula and of the Factors Involved in its Construction; its Usefulness and its Limitations ...	32
IV.	The Results of Applying the Formula in West Africa to Estimating (1) The Relative Importance of *A. costalis* and *A. funestus* as Vectors of Malaria, (2) The Relative Infective Densities in Different Localities...	35
V.	Seasonal Variations in the Infective Densities in Different Localities...	37
VI.	The Estimation of (1) The Inoculation Rate and (2) The Individual Inoculation Risk ...	38
VII.	The Relationship of the Anopheline Infective Density to the Human Malaria Infection Rate in a District ...	42
VIII.	Summary and Discussion ...	47

I. INTRODUCTION

No tropical disease has received more attention than malaria, and a considerable literature is available in every country where it occurs regarding the amount of human malaria, and, though to a lesser extent, the number of and infection rate amongst the local anophelines. Yet it is usually impossible to calculate from the data given one of the most important points regarding the disease—that is, the relative concentration of infective anophelines in different localities or amongst different species.

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*Most of the figures quoted in the following paper have been taken from a previously published article on a mosquito survey of Freetown and Kissy (Gordon, Hicks, Davey and Watson, 1932), and, with the exception of those other figures for which a specific reference is given, the source will not again be referred to.

In a recent mosquito survey of Freetown and Kissy, Sierra Leone, information was collected regarding all the points which at the time were considered to have any bearing on the rôle of anophelines locally in the spread of malaria. As a result of the survey it was found possible to draw a comparison between the anopheline density and infection rate in the village of Kissy and in the city of Freetown, and it was shown that the anopheline density was 34 times as great in Kissy as in Freetown, while the all-species infection rate remained similar. Again, it was suggested that in Kissy *A. costalis*, owing to its higher infection rate, was possibly of more importance than *A. funestus*, although the latter outnumbered this species numerically. After the publication of this paper, we came to the conclusion that, with the detailed information collected regarding some 5,000 anophelines captured and 3,000 dissected, it should be possible to translate these somewhat indefinite statements into precise figures. We now believe that this can be done by combining the figures for the anopheline density and infection rate, and thus estimating the average number of infective anophelines (i.e., anophelines with sporozoites in the glands) per room per night—that is to say, the density of infective anophelines. The present paper is published in the hope of inducing other workers to include in their surveys these two figures which are essential to the calculation of infective density, and therefore to the estimation of the risk of inoculation with malaria.

For the purpose of the present paper, we propose to consider malaria as a disease solely contracted in the house. In every country a certain number of infections are undoubtedly contracted out of doors, and in some countries this outdoor risk is high ; on the whole, however, the vast majority of infections are acquired indoors, and it seems probable that the general concensus of opinion amongst malariologists is in agreement with Clayton Lane (1931), who, having reviewed the literature concerning ' Housing and Malaria,' concludes his article with the words : ' These various lines of investigation press with cumulative force the conclusion that the house is a factor of primary importance in the acquisition and spread of malaria.' In Sierra Leone, and in West Africa generally, the risk of outdoor infection is very small. In a previous report on a mosquito survey (Gordon *et al.*, 1932), it was shown that *A. costalis* and *A. funestus* were almost the sole vectors of malaria throughout West Africa, and that both were essentially house-haunting species and were seldom captured biting out of doors. These observations have been continued and confirmed, only 35 *A. costalis* and 17 *A. funestus* being captured biting out of doors in August, during a period of 100 boy hours. At the same season of the year, the anopheline density in houses, as reported in the paper quoted above, varied between 10 and 20 per room per night. These findings confirm those of Barber and Olinger (1931) in Nigeria, and show that in West Africa, though outdoor biting anophelines must play some small part in the transmission of malaria, yet the infective density, as calculated from the collections in houses, represents for all practical purposes the number of infective anophelines occurring in the district.

II. PREVIOUS METHODS OF CALCULATING THE ANOPHELINE INFECTIVE DENSITY

Ross (1910) proposed a formula for estimating the risk of malaria infection which requires knowledge regarding both the number of gametocyte carriers and the number of anophelines in a district. The final condensed expression of his formula is that ' the inoculation rate per hundred of population equals about half the malaria rate [i.e., the percentage of persons who contain plasmodia at some given moment] multiplied by the mosquito ratio [the number of anophelines to each human being].' The full formula of which this is an approximate abbreviation is, however, affected by two factors, both of which are assumptions, and, as Ross himself states, ' are conjectured on a basis of general probability, not determined by investigation.' In this method Ross substitutes for the actual anopheline infection rate based on dissections, an estimated anopheline infection rate based on the ratio of the numbers of anophelines to the numbers of gametocyte carriers in the district. Waite (1910) also treats mathematically the data supplied by Ross's report on malaria in Mauritius, but here again numerous assumptions are necessary. Further, both these methods introduce an unnecessary inaccuracy, since the anopheline infection rate can be obtained as a result of dissection.

At the present time, the amount of malaria amongst the anopheline population, and therefore awaiting distribution amongst the human population in any district, is only vaguely estimated by considering the figures for the anopheline density and the anopheline infection rate. Obviously, if both are high, then the inoculation rate will also be high; if both are low, the inoculation rate will also be low; but if one is high and the other low, the reader is left to draw his own conclusions regarding the 'malariousness' of the district, while only too often but one of these two factors is quoted. Clearly, an estimation of the number of anophelines occurring in a district is by itself of little value: for example, in certain localities in England there exists a higher anopheline density than in some malarious districts in the tropics. Again, a high anopheline infection rate in one district does not necessarily indicate that there is a greater risk of contracting malaria than in another district where the anopheline infection rate is low; in parts of India the anopheline infection rate is extremely low, but the great density of anophelines compensates for this, and a high rate of human malaria results. These facts may appear self-evident; yet in the recent literature in many instances only one of the two essential factors is quoted. Even when both figures are given, it may still be difficult accurately to estimate the density of infective anophelines until they are reduced to a single term. We may here discuss two exceptions to the foregoing criticism, where attempts have been made to combine the anopheline density and the anopheline infection rate and express them as a single figure. Anderson (1931), in a small survey carried out in Southern Nigeria, estimated what he refers to as 'the infective danger factor' in several localities 'by multiplying the mean quarterly intensity by the quarterly infectivity.' The formula used by Anderson has this disadvantage, that under the infection rate he includes gut infections together with salivary gland infections—therefore his result does not refer to the possible number of infective bites received (the real infection danger); the actual infective danger factor in the districts examined by him would be lower than the figure he gives, calculated on the general infection rate amongst anophelines. Manalang (1931) has proposed a simple method of calculating the inoculation rate. Using a trap house with human bait, he estimated the anopheline density as the number of anophelines entering the trap during monthly periods. He defined 'infective density' as the ratio of sporozoite infected anophelines to the total anophelines captured in the period, expressed as one salivary gland infected anopheline amongst so many anophelines per month. For the purpose of comparing together different districts or seasons he calculates, on the basis of the factors quoted above, the average length of time during which a person would have to live in a trap in a particular district in order to receive one infected bite. Manalang's method would appear to suffer from the following defects: (1) The use of a single trap house restricts the observation to one small area, and the samples of anopheline density and infectivity obtained might not be representative of the whole locality under consideration. Whereas in many districts, where the anophelines tend to leave the house after feeding, a trap would give more accurate information than an examination of the houses in the early morning, yet in West Africa on the whole the anophelines show little such tendency, and an examination of a number of houses of different types in a given area gives a more correct figure for the density of anophelines in that district than does a single trap house. (2) The factors used in his formula are not always clearly defined. Thus, in estimating anopheline density, no reference has been made to the fact that male anophelines must not be included, since these do not represent potential vectors of malaria, and hence must be excluded from any calculation of the density of infective anophelines. Again, unless every mosquito collected is dissected (usually a difficult object to achieve), some allowance must be made for those not examined; no reference to any such allowance is made by Manalang. (3) It would appear to be unnecessary when using a trap to calculate the infective density as defined by Manalang, i.e., the ratio of infective anophelines to the total anophelines caught. The real density of infective anophelines, that is, the number of infective anophelines in a given area—in this case the trap house—may be more simply stated as the number of infective anophelines which entered the trap during the month, for, from the point of view of immediate risk of malaria inoculation, it obviously does not matter how many uninfected anophelines entered the trap.

III. DEFINITION OF THE PROPOSED FORMULA AND OF THE FACTORS INVOLVED IN ITS CONSTRUCTION; ITS USEFULNESS AND ITS LIMITATIONS

Before it is possible to discuss the malaria inoculation rate, which will be considered in another section, it is necessary to calculate the density of infective anophelines, which is the main factor influencing and, for purposes of comparison, representing the inoculation rate.

If it were possible to catch and examine every anopheline that entered a particular room during 24 hours, then the number of female anophelines captured would represent the anopheline density, and the number of these infected in the glands the anopheline infective density in that room for that day. But the number of anophelines entering varies in the same district in different houses and at different seasons, so that, in order to assess the average anopheline density and infective density, it is necessary to take samples not only from different houses but at different seasons of the year. The figure thus obtained represents the average number of female anophelines found at the time of examination of the houses in the early mornings; but, in order to calculate the number of female anophelines which were present in the houses during the night, it may be necessary to increase this figure by adding to it a previously estimated amount representing the number of anophelines which had left the houses before the time of their examination; thus it is suggested in the previous report that the small number of anophelines found in the early morning in bright airy houses in Freetown is probably due to a proportion of them leaving such dwellings before the time of search, rather than to their disinclination to enter such houses. Although in many localities the proportion of anophelines thus leaving the houses is probably sufficiently large to affect appreciably the density, we do not think it is so in the case of our figures for Freetown and Kissy, where the majority of houses searched were of the small dark type, from which we believe few mosquitoes fly out before dawn. The figures quoted, therefore, for the density in Freetown and Kissy probably fall little short of the actual density during the night. In the case, however, of localities where it is known that the proportion of mosquitoes leaving before dawn is significantly large, allowance must be made for it, and this allowance is expressed in our formula under the term 'x'. We see no reason why 'x' should not be calculated, by means of traps, etc., but in Sierra Leone our attempts to do so have so far been unsuccessful.

The estimation of the anopheline density is simple, and since more than one house is examined the following formula is used:

$$\frac{\text{Total number of female anophelines captured} + \text{'}x\text{'}}{\text{Total number of rooms examined.}}$$

Again, since it is often impossible to dissect all the mosquitoes captured, the anopheline infective ratio is estimated from the formula:—

$$\frac{\text{Total number of sporozoite infected anophelines}}{\text{Total number of female anophelines dissected.}}$$

By multiplying together the anopheline density and the ratio of infective anophelines, we obtain the average number of infective anophelines per room per day, i.e.,

$$\frac{\begin{array}{c}\text{Total number of female}\\ \text{anophelines captured} + \text{'}x\text{'}\end{array}}{\begin{array}{c}\text{Total number of rooms}\\ \text{examined}\end{array}} \times \frac{\begin{array}{c}\text{Total number of sporozoite}\\ \text{infected anophelines}\end{array}}{\begin{array}{c}\text{Total number of female}\\ \text{anophelines dissected}\end{array}};$$

—this equals the density of infective anophelines, that is to say, the number of sporozoite inoculations awaiting distribution in each room each night, which in future we propose to refer to as 'The Anopheline Infective Density,' or briefly 'The Infective Density.'

The formula thus given represents the actual infective density, and if the term 'x' is known it will enable us to compare together the anopheline infective densities of localities in different parts of the world, so long as the species transmitting malaria are indoor biters. *What we wish to emphasise at this point is that when comparing together infective densities, so long as the anophelines, whether of one or more species, behave similarly as regards the period of time they remain in the house after their blood meal, the modifying term* ' x ' *does not require to be taken into consideration, as it is a factor common to both the infective densities.* We have shown that in West Africa only two species are concerned with the transmission of malaria, and we believe that all the literature concerning these species, *A. costalis* and *A. funestus*, tends to show that they are essentially house-haunting species and possessed of the same habits, so that in comparing together infective densities in West Africa the term '*x*' may be omitted from consideration.

We believe that the use of this method of expression is of considerable practical value; for example, a Medical Officer has the choice of two sites for occupation by troops, both of which appear to be equally suitable, but previously obtained figures show that in one of the localities 2,000 female anophelines of certain species were captured in 80 rooms, 1,500 of these were dissected and 90 found to be infected in the salivary glands. In the other area, 3,000 anophelines of the same species were taken in 250 rooms and 200 found to be infected with sporozoites in a total of 2,500 dissected. In which area is the inoculation risk greatest? Expressed as infective density, the relative risk in the first district is :—

$$\frac{2{,}000}{80} \times \frac{90}{1{,}500} = 1{\cdot}50.$$

In the second district the corresponding figure is :—

$$\frac{3{,}000}{250} \times \frac{200}{2{,}500} = 0{\cdot}96.$$

In short, the first district contains about 50 per cent. more infective anophelines than the second. Again, its value to the Medical Officer of Health is equally obvious, for by means of it he can chart the progress effected by anti-malaria measures over varying periods. From the same point of view, it allows an estimation to be made of the part played by different species of anophelines in the local transmission of malaria, a matter of importance nowadays when general anti-larval measures are tending to give place to more specific methods directed against individual species.

As we have already pointed out, the accuracy of the results obtained by this method will be affected by the size and thoroughness of the survey carried out. No law can be laid down as to the number of houses which should be examined, the same rules apply as in mosquito surveys generally and they vary from place to place. Again, in West Africa and elsewhere, marked seasonal variations occur in anopheline density and to a less extent in the infection rate, so that the ideal to be aimed at is a 12 months' observation period.

IV. THE RESULTS OF APPLYING THE FORMULA IN WEST AFRICA TO ESTIMATING (1) THE RELATIVE IMPORTANCE OF *A. COSTALIS* AND *A. FUNESTUS* AS VECTORS OF MALARIA, (2) THE RELATIVE INFECTIVE DENSITIES IN DIFFERENT LOCALITIES

We have already shown that in West Africa only two species of anophelines (*A. costalis* and *A. funestus*) are concerned in the house transmission of malaria, and we believe that they behave similarly as regards their length of stay in a house after a blood meal. Therefore, in comparing infective densities in West Africa, the term '*x*' (representing the proportion which leaves a house before dawn) is omitted from our calculations.

(1) *The relative importance of* A. COSTALIS *and* A. FUNESTUS *as vectors of malaria.* In the report on the mosquito survey of Freetown and Kissy, figures were given for the total anophelines captured, together with the number per room and the percentage found infected in the glands. In Freetown *A. funestus* did not occur in appreciable numbers, while in Kissy the following data regarding the two species were obtained :—

Rooms examined	Species	Female anophelines captured	Rate per room	Total dissected	Percentage infected with sporozoites
582	*A. costalis* ..	1,630	2·8	1,157	11·1
	A. funestus ..	1,938	3·3	907	4·1

All the necessary figures for calculating the infective density are thus available; but as the sporozoite infection rates are here expressed as a percentage, it is necessary to divide by 100 to obtain the anopheline infective ratio, so that these figures become 0·111 and 0·041 respectively. We can now express in a single term the infective density of *A. costalis* as 0·311 and of *A. funestus* as 0·135; that is to say that at Kissy, averaged over a period of 12 months, although there were only 28 *A. costalis* in every 10 rooms as compared with 33 *A. funestus*, there was one infective *A. costalis* in every third room every night, whereas there was only one infective *A. funestus* in every eighth room. Further, we can also state that, since these two species are the only anophelines occurring in significant numbers at Kissy, *A. costalis* is responsible for some 70 per cent. of the malaria transmission there, as compared with 30 per cent. by *A. funestus.*

(2) *The relative infective densities in different localities.* Since Freetown represents a locality in which systematic anti-anopheline measures have been carried out on a gradually increasing scale for some 30 years, and since the neighbouring native village of Kissy differs but little from any unsanitated village anywhere in the Colony, it is of particular interest to compare the infective densities in these two localities. The anopheline survey of Freetown and Kissy has shown that in the wet season the combined anopheline rates were as follows :—

	Freetown	Kissy
Total rooms examined	3,005	320
Total female anophelines caught	1,201	3,340
Total female anophelines dissected	1,153	1,855
Total female anophelines with sporozoites	70	150

Applying the formula as in our previous examples, we obtain the following results : infective density in Freetown 0·0242, and in Kissy 0·844. That is to say, the infective density in the unsanitated native village of Kissy is 34 times as great as in the neighbouring sanitated city of Freetown, a concise expression of the results of prolonged anti-mosquito measures.

In West Africa generally, few records of both the anopheline density and the anopheline sporozoite rate are available. Barber and Olinger (1931) published figures which enable us to estimate the infective densities* in two areas in Nigeria (Lagos and its suburbs, and Ibadan) and to compare them with the infective densities in Freetown and Kissy. All four figures quoted below are for the same period of the year and all species are included. In Freetown and Lagos, *A. costalis* represents nearly 100 per cent. of the total anophelines captured ; while in Kissy and Ibadan, *A. costalis* and

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* The figures given by Barber and Olinger for the anopheline density may include a certain proportion of male anophelines, but probably these latter do not r present more than 10 per cent. of the total catch. The infective density will therefore be slightly lower than the figure estimated.

A. funestus together comprise almost the entire anopheline collections The anopheline infective densities in the various towns were found to be as follows :—

Freetown	0·024
Ibadan	0·098
Lagos and suburbs	0·403
Kissy	0·844

We have already shown that the anopheline infective density in Kissy is 34 times as great as in Freetown, and it can now be seen that in Ibadan it is 4 times, and in Lagos 17 times, as great.

V. SEASONAL VARIATIONS IN THE INFECTIVE DENSITIES IN DIFFERENT LOCALITIES

It is well recognised that the risk of contracting malaria varies in most localities at different seasons of the year, usually bearing some relationship to the rainfall, and we have shown that it must be directly proportional to the anopheline infective density. This variation will be dependent on changes in the anopheline density, in the anopheline infective ratio, or in both these factors.

In Freetown, anophelines were so rare in the dry season that workable figures were not obtainable ; almost certainly the anopheline density did not exceed 0·02 per room with an infective ratio of not more than 0·06 ; that is to say, the infective density during the dry season is below, and probably very much below, 0·0012. In the rains the infective density is shown to be 0·024, that is to say, 20 times as great. Expressed in a more graphic form, there is one infective anopheline nightly in Freetown to every 833 rooms during the dry season, and one to every 42 rooms during the rains. This figure may seem fantastically low, and we have stated that it probably is slightly below the actual figure, but we believe it is only slightly so, and when we compare it with the figures for villages in an unsanitated condition, searched with exactly the same technique, we observe that they present a very different picture throughout the year and particularly during the dry season.*

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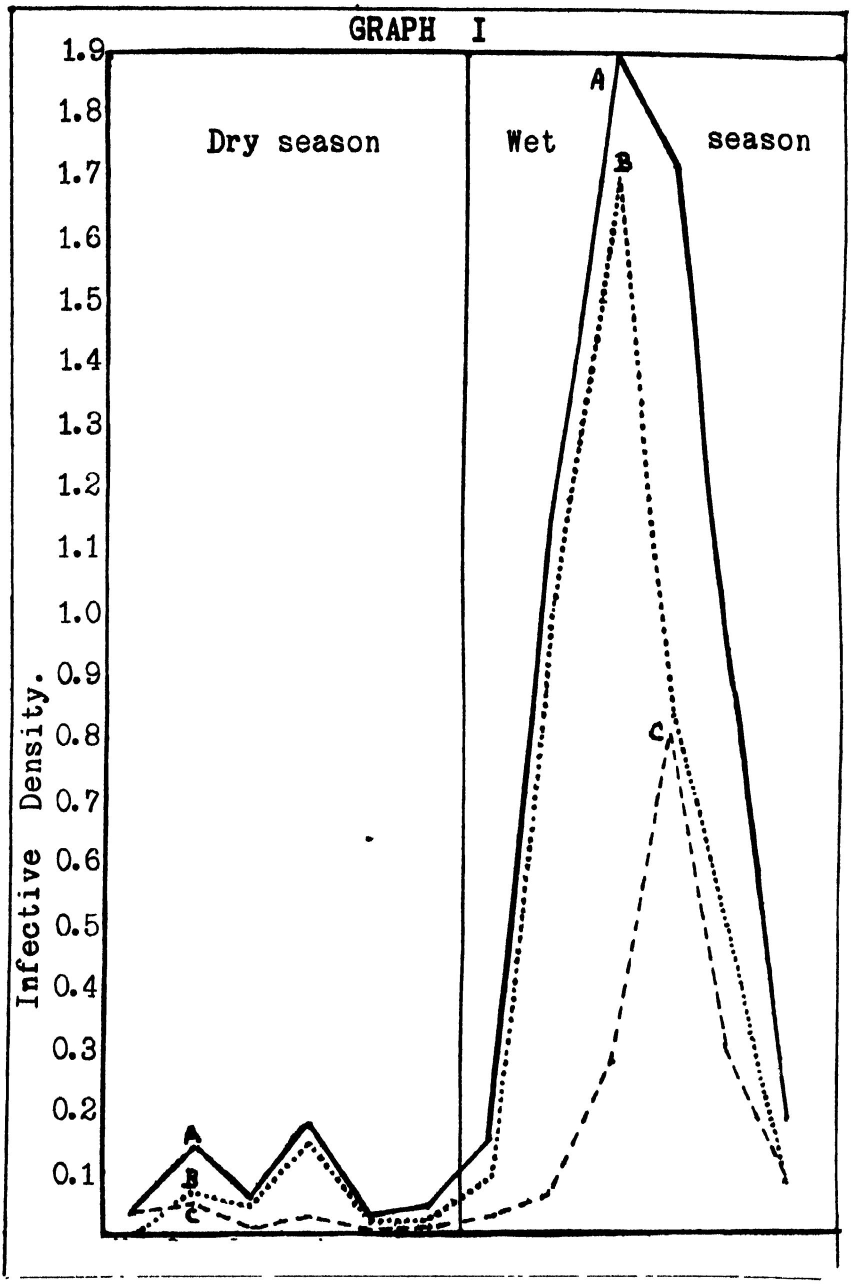
* That the infective density in European ' bush camps ' may rise to astonishing heights even in the dry season, when the camp is in the vicinity of a native village, was impressed on us during a three weeks' survey in March, 1931, of a temporary mining camp. In this camp the anopheline density, estimated by daily examinations of some 10 houses, was 2·9, and the infective ratio based on 250 anophelines dissected was 0·141, giving an infective density of 0·41.

In Kissy, our figures are adequate for the whole year, and below, in Graph I, we show the seasonal variation in the anopheline infective density and the parts played by *A. costalis* and *A. funestus* in determining it. The total anophelines captured in Kissy during the 12 months' observation period were *A. costalis* 1,630, *A. funestus* 1,933, *A. nili* 27 and *A. rhodesiensis* 19. Thus the numbers of species other than *A. costalis* and *A. funestus* were too small to allow of individual graphs being drawn. None of the *A. rhodesiensis* captured were infected, so that their infective density is 0.

From Graph I it can be seen that a very striking difference existed in Kissy between the infective densities in the wet and the dry seasons, the average dry season figure for all species being 0·075, or one infective anopheline in every thirteenth room, and during the rains 0·84, or 10 infective anophelines to every 12 rooms. At the onset of the rains, there was an immediate rise, culminating in a maximum of 1·9 in July (i.e., almost 2 infective anophelines per room per night), but this high rate was only maintained for another month, after which a steady and rapid fall in the infective density ensued. It can also be seen that *A. costalis* was almost entirely responsible for maintaining the infective density throughout the year; *A. funestus* only affected markedly the general infective density in July, August and September, and during these months only rose to a maximum of 0·8, a point of considerable interest in view of the fact that during August, September and October, *A. funestus* numerically predominated over *A. costalis* in the proportion of approximately 3 to 1.

VI. THE ESTIMATION OF (1) THE INOCULATION RATE, AND (2) THE INDIVIDUAL INOCULATION RISK

(1) *The inoculation rate.* Up to this point we have been dealing with facts capable of direct calculation, and have shown that the relative infective density can be estimated with a correctness the degree of which is dependent on the extent of the survey, so that the infective densities in different localities or of different species may be compared together, so long as the species in question possess similar habits. Thus, throughout West Africa, where the transmitting anophelines are always of the same two species, and where both



Graph I

Showing the seasonal variation in anopheline infective density for all species at Kissy (A), and the parts played in determining it by *A. costalis* (B), and *A. funestus* (C).

species possess similar habits, the infective densities of any localities and of the two species may be compared together with considerable accuracy. We have further suggested that when comparing districts where the habits of the anopheline vectors are dissimilar, comparisons may still be drawn by making allowance for the proportion of anophelines in each district which leave the house before the time of its examination in the morning.

We have defined infective density as representing the number of sporozoite inoculations awaiting distribution in each room each night; similarly, we propose to define the inoculation rate as the number of sporozoite inoculations distributed in each room each night. Since the inoculation rate must vary directly with the infective density, this latter is the only calculation necessary when comparing different localities. It is, however, of some interest to estimate what fraction of the infective density represents the inoculation rate, for it seems reasonable to assume that it may be estimated with a considerable degree of accuracy. Unfortunately, up to the present time, we have failed to collect precise information on two factors which are necessary to its exact determination, and we therefore have to introduce certain hypotheses. If every infective anopheline that entered a house fed the same night, then the inoculation rate would exactly equal the infective density, but it has been shown (as a result of examining the guts of 600 anophelines from different houses in Kissy) that 92 per cent. were fed and showed evidence of having partaken of a blood meal within 48 hours previous to capture. Unfortunately, so far we have been unable to determine more accurately the time of feeding, so that we cannot say what proportion of these fed anophelines had taken blood on the night prior to capture, and what proportion during the 24 hours previous to that. It is reasonable to assume that only a proportion had fed during the night before capture, for as a result of diffusion experiments we concluded that the anopheline population in Sierra Leone changes nightly in every house, and that many anophelines probably enter another house before digesting their blood meal and with no desire for a further feed that night (Gordon *et al.*, 1932). It is obvious that all the infective anophelines captured in a room or an area will be considered in the infective density, but it is equally obvious from the results quoted above that only a proportion of these will represent bites that have actually been distributed the previous night, and due allowance must be made for this fact. Further than this, a certain number of anophelines will bite more than once owing to 'interrupted feeding,' and hence the actual number of bites distributed would be greater than the number estimated on the basis of one bite per infective anopheline. Let us assume that one-half of the anopheline collections in the morning had fed on the night previous to capture, this proportion to include the interrupted feeds. Then to obtain the inoculation rate it is necessary to divide the infective density by two. Thus, during the wet season in Freetown the inoculation rate would be $\frac{0{\cdot}024}{2} = 0{\cdot}012$, that is to say, one infective mosquito bites in every eighty-fourth room per night; in Kissy, during the same period, the rate would be 0·42 (approximately 1 in every 2½ rooms), and over the whole year 0·21 (that is 1 in every 5 rooms).

It is clear that this calculation of the inoculation rate is based on an assumption, and probably only approximates to the actual figure; in the same way, the individual inoculation risk, since it is based on the inoculation rate, will be subject to the same error.

(2) *The individual inoculation risk.* We use the term 'inoculation risk' in preference to the more usual term 'malaria risk,' as the former is capable of direct calculation, while in the case of the latter the personal factor (immunity, prophylaxis, etc.) renders it incalculable, and we define it as the number of sporozoite inoculations received by each individual in each room each night. If the inoculation rate in a room is, for example, 1·0, and only one person is sleeping in that room, then that individual receives one infective bite per night; but if two persons share the room, then the risk is divided between them, and on the average the risk of sporozoite inoculation for each individual is half the inoculation rate; so that, in attempting to estimate the individual inoculation risk in any locality, we must take into consideration the average density of the human population per room. It is unfortunate that at present we are not in a position to give more than an approximate figure for the inoculation rate, since the individual inoculation risk is directly and accurately calculable from it, so long as the number of occupants of the sleeping rooms is known, and this figure we have obtained for all the houses searched for anophelines in Freetown

and Kissy. In an examination of approximately 600 houses in Freetown, the population density was found to be 1·9 per sleeping room, so that the individual risk in Freetown is equal to half the inoculation rate. In Kissy, a rural community, the population density is slightly higher on the average, and in some individual cases is extreme, the average being 2·2 per room, and in one three-roomed house 4·6 per room. On the basis of these figures, the individual inoculation risk may be calculated for Freetown as being 0·0063 during the rains, and in Kissy, during the same period, as being 0·19, some 30 times as great as in Freetown.

How great is the risk of infection and re-infection with malaria run by every occupant of a house with a high anopheline infective density is shown by the following account of a house in Kissy where the anopheline infective density was unusually high. In this house, where adequate figures were obtained, the anopheline density for the whole year averaged 23·6 per room, varying from 2·6 to 45 per room, and the infective density for the year was 3·17. If, as before, we assume the number of infective anophelines biting to be 50 per cent. of the total caught, then the inoculation rate would be 1·58, and as there were 9 people (6 adults and 3 children) inhabiting 3 rooms, then the individual infection risk would equal 0·53. That is to say that, averaged over the whole year, each person in that house would receive an infected bite every other night, or approximately 193 malaria infected bites in the year.

We have proved that the individual inoculation risk in Kissy is many times as great as in Freetown. Is it reasonable to expect that a marked difference exists between the human malaria rates in these two localities ?

VII. THE RELATIONSHIP OF THE ANOPHELINE INFECTIVE DENSITY TO THE HUMAN MALARIA INFECTION RATE IN A DISTRICT

It has been shown that during the wet season in Freetown the average number of infective bites received per person is probably less than 1 every 3 months, and that during the dry season the individual inoculation risk falls far below this ; yet in a previous paper (Gordon and Davey, 1932) we showed that the human infection rate amongst 200 native children aged from 3 to 14 years was over

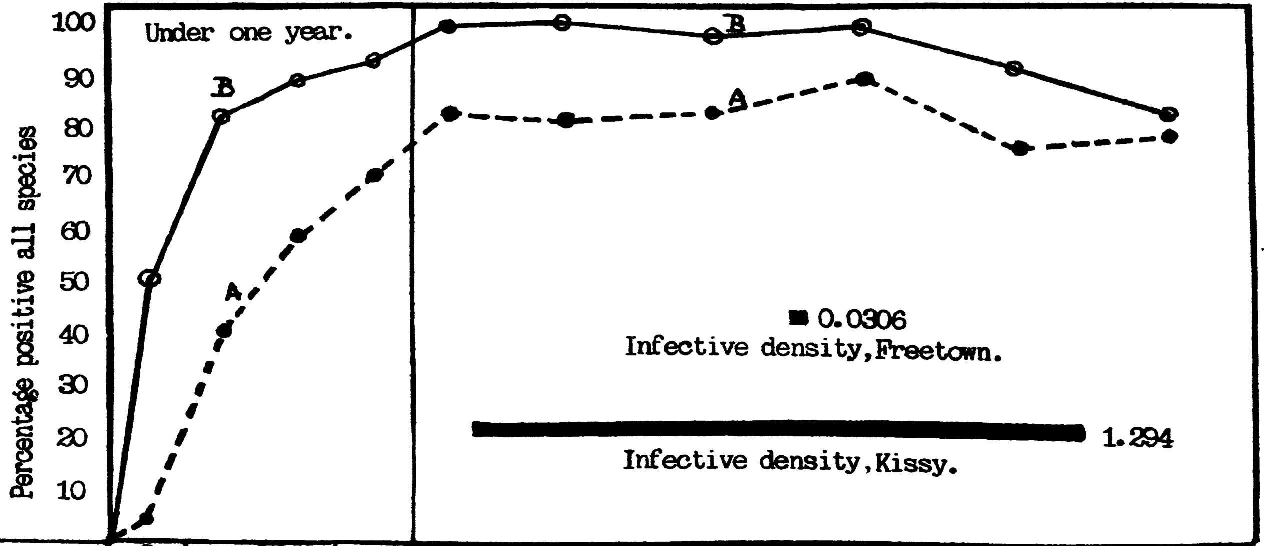
50 per cent. when examined during the dry season. Again, during the present investigation, we examined 280 school children in Freetown and a similar number in the native village of Kissy during the height of the wet season ; in the former the infection rate was found to be 81 per cent. and in the latter 92 per cent., a very small difference in the human malaria rate when it is remembered that the difference in the anopheline infective densities in the two districts was as 1 to 30. In Freetown, therefore, the examination of the human population suggests a highly malarious district, whereas the examination of the anopheline population would, on the contrary, indicate a very low malaria risk. We believe that this lack of correlation between the infective densities and the proportion of school children found infected with malaria in the two districts is due to the fact that fresh infections are not usually followed by complete recovery, but that the parasites reappear intermittently in the peripheral blood. Thus, the great difference between the small number of fresh infections occurring per person in Freetown and the large number occurring in Kissy becomes lost in the mass of relapses common to both districts ; we think, therefore, that the relapse rate is very much higher than the primary infection rate. Where, however, as in Kissy, the infective density is very high, the significance of the relapse rate is not noted, because already a high primary infection rate must exist, and an additional relatively small proportion of relapses is sufficient to produce a malaria incidence of nearly 100 per cent. ; in short, we cannot by examining the human population distinguish between saturation and supersaturation. With our present lack of knowledge regarding the duration of the primary attack and the number and duration of the subsequent relapses, the estimation of the malaria incidence in the general population is of little value as an indication of the malaria inoculation risk in a community.

If, therefore, we wish to ascertain what correlation exists between the anopheline infective density and the human malaria rate, we must conduct our investigations amongst that section of the population that is exposed to infection for the first time ; that is to say, the non-immune newcomer to the district, or else the infant population during the first few months of life.

If this theory is correct, then the great difference in the anopheline infective densities in Freetown and Kissy, although its effects are not reflected in the malaria incidence amongst the older school children in these localities, should be clearly demonstrable in the respective malaria infection rates of the children of less than one year old. The ideal method of testing this theory would be to examine a number of children in both localities at short intervals from birth onwards, in order to discover at what average age period the first infection occurred amongst the infants of each community. This method was not at the time practicable, and instead the plan we adopted was to examine simultaneously during the most intensely malarious months of the year, both as regards the human infection rate and the anopheline infective density, as many infants under a year old as were available in two communities in which the anopheline infective density for the same season of the year had already been ascertained. In Kissy, at this time of the year, the known infective density was 1·29, and the total number of children available under a year old was 82 ; in Freetown, the infants were selected from the same area as that in which we had previously ascertained that the anopheline infective density was 0·031. Here again the number of children of a suitable age was limited, as a prophylactic quinine campaign was at the time in progress amongst infants in this hyperendemic area, and all children who had received quinine had to be excluded ; in all, we examined 72 infants in Freetown under a year old. The children in the two localities were divided into 4 three-monthly age periods, and the results of their blood examinations are compared below in Graph II. In order to compare accurately the age incidence of malaria amongst those older children in Freetown and Kissy, the general infection rates amongst whom have already been discussed, the results of their examination are also included in the graph in 5 two-yearly age groups. It was unavoidable that the total number of cases examined was small, but the regularity of the graphs obtained appears to prove that the results represent those that would have been obtained from larger figures.

The comparison of the infection rates in Kissy and Freetown amongst children from birth to 12 months, as shown in Graph II, would appear to prove the truth of our hypothesis, that the difference in the anopheline infective densities of two localities will be reflected

GRAPH II



Age		3	6 months	9	12	2	3 | 4	5 | 6	7 | 8 years	9 | 10	11 | 12
Number examined	F'town	21	17	17	17	43	22	107	61	38	38
	Kissy	31	19	19	13	29	10	41	56	50	46

GRAPH II.

Showing the incidence of malaria at various age periods from birth to 12 years amongst 381 children examined at Freetown, Graph A, and 285 children examined at Kissy, Graph B, during September and October, which are probably the most malarious months of the year. For comparison, the relative infective densities of the two localities during the same period are indicated by horizontal columns.

in the human infection rates during the earliest months of life, and this difference will become less as the child grows older. Thus in the first 3 months of life of children living in the high infective density of 1·29 in Kissy, no less than 52 per cent. were found infected on the day they were examined, whereas in Freetown, where the child lives in the comparative freedom from inoculation represented by an infective density of 0·031, only 5 per cent. showed parasites. With each few months of advancing age, this original great difference steadily diminishes ; thus, in the six-months age group, the infection rates in Kissy and Freetown are respectively 83 per cent. and 41 per cent., in the nine-months age group 89 per cent. and 59 per cent., while in the twelve-months age group they are 93 per cent. and 70 per cent. respectively. The occurrence of relapses in regularly increasing numbers appears to be the most reasonable explanation of the fact that this difference in the infection rates of the two localities steadily decreases from 3 months onwards, so that from 10 months to 12 years, the limit of our observation period, an average difference of about 15 per cent. is more or less steadily maintained. This figure certainly does not represent the great difference in the anopheline infective densities of the two localities, although, as we have already pointed out, once the infection rate reaches 100 per cent., it becomes impossible to differentiate between saturation and super-saturation.

We can, therefore, at this point answer our own question of the previous section as to whether it is reasonable to expect that a great difference in the anopheline infective densities in two localities will result in a marked difference in the respective malaria rates amongst their inhabitants. The answer appears to be that little difference may be expected in the general infection rate, but that a great and maximum difference will be found in the respective birth to three-months age groups.

As already stated, the only figures available from which anopheline infective densities in West Africa can be calculated are those of Barber and Olinger (1931) and Anderson (1931). The latter gives no relevant figures regarding the malaria infection rate in his locality, but the former give very extensive and precise estimations of the human malaria rate for various age groups in Nigeria, most of these figures being derived from children examined in Lagos and its suburbs, a district in which we have already shown the anopheline infective density to be intermediate between those noted in Freetown and Kissy. One would expect, therefore, that a graph of the infection rate amongst children of age groups similar to those examined in Freetown and Kissy would fall intermediate between the graphs depicted for Freetown and Kissy. That it actually would do so to a remarkable extent is shown by the figures quoted in the Table below ; in spite of this, for various reasons we consider that too much significance should not be attached to this additional proof of the relationship between infective density and the parasite rate in young infants. Whereas our figures represent simultaneous examinations in the two most malarious months of the year amongst children living in a limited area where the anopheline infective density was known, Barber and Olinger's figures for the anopheline density and infection rate (from which we have calculated the infective density) represent an average over a very large area, but we have no means of knowing that the infection rate amongst the children is averaged over the same area.

The correlation of these three sets of figures with their respective infective densities is remarkable, and it will be noted how small are the differences in the figures for malaria incidence amongst the older age groups of the children examined.

With only three sets of figures at our disposal, it would be unwise to postulate any definite ratio between the anopheline infective density and the primary infection rate amongst young children. The ratio will probably be found to be in the nature of a steady progression, that is to say, if they were graphed against each other the product would probably tend to follow a straight line.

VIII. SUMMARY AND DISCUSSION

We believe that in the present paper the following points have been demonstrated :

(1) The estimation of the density of infective anophelines is a matter of importance to any community in which malaria exists. The method of calculating it, which we have described, has certain advantages over the few formulae previously suggested : it is practicable, and involves no collection of data other than those readily available during the course of a routine anopheline survey.

Table showing the malaria infection rate noted amongst children living in three towns in West Africa where the anopheline infective density was known. The figures for Lagos and suburbs are quoted from Barber and Olinger (1931).

Locality	Freetown	Lagos and its suburbs	Kissy
Anopheline infective density ...	0·031	0·61	1·29
Ages	Percentage positive	Percentage positive	Percentage positive
1 month	4·8	25·0*	51·6
2 months		..	
3 ,,		22·4	
4 ,,	41·2	29·7	84·0
5 ,,		57·6	
6 ,,			
7 ,,	58·8		89·5
8 ,,			
9 ,,			
10 ,,	70·6		92·2
11 ,,			
12 ,,		90·0	
2 years	83·7	93·6	100
3 ,,	81·8	98·2	100
4 ,,		93·6	
5 ,,	82·5	93·7	97·6
6 ,,			
7 ,,	90·0		100
8 ,,			
9 ,,	76·3	86·7	92·0
10 ,,			
11 ,,	79·0		82·6
12 ,,			

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* This figure is incompatible with Barber and Olinger's other figures, and is founded on the examination of 16 children.

(2) In districts where house-haunting anophelines with similar habits occur, as is the case throughout the whole of West Africa, a knowledge of the anopheline infective density enables an estimate to be made of the relative importance of each species of anopheline vector in the district. It further enables a comparison to be drawn between the relative infective densities of different districts. Armed with this knowledge, the sanitarian can concentrate his efforts on the particular district most urgently in need of attention, and can record accurately the results that follow the measures adopted; moreover, he knows beforehand against what species of anophelines his work must be directed. For example, in a village in Sierra Leone, where the only significant vectors were *A. costalis* and *A. funestus*, we found that *A. funestus*, in spite of its numerical superiority over *A. costalis*, was only responsible for some 30 per cent. of the malaria transmission. Again, the effect of prolonged anti-mosquito measures is to be seen in the fact that the infective density in Freetown is to-day only one-thirtieth of that in a neighbouring unsanitated village, which we believe closely resembles the Freetown of some 30 years ago.

(3) Since great changes occur in the anopheline infective density at different seasons of the year in West Africa, and almost certainly in other countries, when estimating the infective density it is advisable to average this figure on the results of a year's observations. Figures are available from a village in Sierra Leone over 12 consecutive months, and they show that in this locality the anopheline infective density during the wet season is 12 times as great as during the remaining 6 months of the year.

(4) Whereas the individual risk of inoculation with malaria cannot as yet be estimated accurately from the anopheline infective density, nor is the exact relationship between the two known, nevertheless the former is dependent on the latter, for, by comparing together the malaria infection rates in different districts, we have been able to prove that a close correlation exists between a high infective density and a high malaria rate amongst children examined during the first 3 months of their life, while a low infective density is similarly associated with a low infection rate.

(5) In contradistinction to this close association, we have shown that an estimation of the malaria rate amongst the older age groups

bears little relationship to the anopheline infective density, a high infection rate being rapidly reached amongst them as a result of re-infection and relapse, and is therefore useless as an indication of the malaria infection risk, or, as it is commonly called, the 'malariousness,' of a district.

It is of interest to note that the age incidence curve of malaria infection in 1932 follows very closely that described by Blacklock and Gordon in 1925, and to compare their explanation of its course with that advanced in the present paper. Blacklock and Gordon suggest two possible explanations of the very low infection rate amongst young infants in Freetown, as follows: 'It would be compatible with the idea that the child at birth was endowed with a passive immunity derived from the mother which steadily diminished until the age of 1½. It would also be compatible with the idea that effective exposure to infection increases steadily as the child grows older until it reaches the age of 1½. The flattening of the curve between the ages of 1½ and 2½ (which is as far as our figures go) would, if the passive immunity theory is accepted, almost certainly be due to the gradual acquirement of active immunity by the child after this age, which increases directly in proportion as the passive immunity wears off. The second explanation appears less probable since it fails to explain the flattening of the curve.' At the time of publication of the above quotation, no data were available regarding the anopheline infective density in Freetown, and clearly the authors were under the impression that this was far higher than was actually the case; the infective density as now established is certainly quite compatible with the figures recorded both in 1926 and 1932. The passive immunity postulated by them seems unlikely, since we have shown that in the village of Kissy no less than 50 per cent. of the children up to the age of 3 months are infected. We believe that the flattening of the curve between 1 and 2 years represents the period at which the combined infections, re-infections and relapses have reached their maximum; in Kissy this maximum is represented by an infection rate of 100 per cent., and in Freetown of 85 per cent.

In view of our knowledge of the relationship between the anopheline infective density and the malaria rate, it is of interest to consider the results of anti-anopheline measures in West Africa. We have little evidence regarding the effects of malaria on the health of the native community at different age periods. It is held by some that tolerance to the infection is only gained by repeated attacks, and that if every native child must, as we believe it must, eventually acquire the disease, then little advantage is to be gained by mere postponement. This view does not take into consideration the fact that it is reasonable to think that a primary attack acquired during the first few months of life is more dangerous than one at an older and more resistant age. Thus, young children in Freetown are rarely attacked by malaria during the first and most critical months of life, whereas in Kissy at least 50 per cent. are so attacked. For various reasons we think that a comparison of the infant mortality in Freetown and Kissy would be unreliable, but there can be little doubt that this postponement of the primary attack must reduce the death rate amongst infants. That it is only a postponement of the disease has, however, already been shown, and as the child grows older parasites are to be found in its blood almost continuously, so that during these later years it is little better off than if it had lived in an unsanitated village. Whatever different views are held regarding the effect of anti-anopheline measures on the health of the native community, no such difference of opinion can exist regarding its value in preserving the health of the European, for, with the aid of personal prophylaxis in a locality with a low infective density, he may be able to ward off malaria indefinitely.

Finally, we must point out that in this discussion we have considered the results of anti-mosquito sanitation with reference to malaria alone, but these measures have in addition in Freetown produced a remarkable diminution in the culicine concentration and probably an important reduction in the diseases transmitted by them.

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A FURTHER NOTE ON THE INCREASE OF *P. MALARIAE* IN FREETOWN, SIERRA LEONE

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In a previous paper (Gordon and Davey, 1932), we recorded that a remarkable rise in the incidence of *P. malariae* had occurred in recent years amongst native infants and school children in Freetown, and in an addendum added three months later we showed that quartan malaria was still increasing.

Now, a year later, we have had occasion again to examine children of similar age groups in Freetown, and find that this increase of *P. malariae* is still being maintained, and that quartan malaria is tending to become the predominant endemic form of malaria in Freetown. As before, this change is most marked in the older age groups, *P. falciparum* still being responsible for the majority of the infections amongst children under 3 years of age. The apparent rise in the general infection rate is probably almost entirely due to the fact that the examination was carried out in the hyperendemic area and at the most malarious season of the year. Thus Macdonald, in 1926, at approximately the same season of the year, examined 337 children aged 3 to 12 years in this same area, and found 72 per cent. infected, which corresponds fairly closely to the 81 per cent. found infected by us in 1932, as shown below.

The figures for 1925, 1926 and 1931 are based on the examination of children coming from all parts of Freetown. When we compare the proportions of the species in the hyperendemic area, as noted by Macdonald in 1926, with those found by us in 1932, the change is even more marked. In 1926 it was found that 57 per cent. of the children were suffering from malignant tertian malaria and 14 per cent. from quartan, as compared with 25 per cent. infected with malignant tertian and 37 per cent. with quartan in 1932.

Table showing the incidence of *P. falciparum*, *P. malariae* and *P. vivax*, amongst native children in Freetown, as noted in various years.

		Already published			
Year of examination		1924-25	1925-26	1931	1932
Number of cases examined	under 3 years ...	809	...	348	230
	3-14 years	...	1,059	821	278
		Percentage positive			
P. falciparum ...	under 3 years ...	18·4	...	23·8	20·0
	3-14 years	...	41·4	16·1	24·8
P. malariae	under 3 years ...	1·5	...	10·9	10·4
	3-14 years	...	7·9	19·6	36·7
Mixed infections with *P. falciparum* and *P. malariae**	under 3 years ...	0·6	...	4·6	18·3
	3-14 years	...	0·3	2·8	18·7
P. vivax	under 3 years ...	0·3	...	1·7	0·44
	3-14 years	...	1·0	0·1	0·72
Total	under 3 years ...	20·9	...	41·1	50
	3-14 years	...	51·1	39·3	81·3

* The percentage of mixed infections in which *P. vivax* occurred is omitted as being negligible.

The above Table shows that quartan malaria is now firmly established in Freetown and is tending to become the predominant type of infection amongst children. How far the rest of Sierra Leone is participating in this change we have not sufficient data to show, but that it extends outside Freetown is suggested by the following figures obtained from Kissy, a native village some 6 miles from the capital. Amongst 112 children under 3 years old, 83 per cent. were positive; 35 per cent. harboured *P. falciparum*, 34 per cent. *P. malariae*, and 11 per cent. mixed infections of the two species. In the 3 to 14 years age group, amongst 227 children 94 per cent. were infected, 20 per cent. with *P. falciparum*, 41 per cent. with *P. malariae*, and 28 per cent. with a mixed infection of both species. The infection rate with *P. vivax* was somewhat higher than in Freetown, the proportion, including mixed infections in the younger age group, being 4 per cent. and, in the older, 6 per cent.

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A YELLOW FEVER PROTECTION TEST IN MICE BY INTRACEREBRAL INJECTION*

BY

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After the discovery of the susceptibility of white mice to the virus of yellow fever (Theiler, 1930), investigators turned to the possibility of devising a method whereby these animals might be used in testing sera for the presence of protective antibodies. By adding to 13 yellow fever immune sera equal quantities of a centrifuged suspension of infective mouse brain and injecting these mixtures intracerebrally into mice, the writer (Theiler, 1931) was able to show protective action in all 13 sera. The amount of protection, as measured by the number of mice that lived or the number that died only after a prolonged incubation period, varied considerably in repeated tests with the same immune sera. These findings were confirmed by Dinger (1931), who emphasised the importance of filtering the virus.

Sawyer and Lloyd (1931) devised a protection test for yellow fever which is known as the ' intraperitoneal protection test in mice.' This test depends upon the fact that when mice are inoculated intraperitoneally with a large amount of yellow fever virus fixed for mice the virus enters the blood stream. If, at the same time, the brains of the mice are damaged by the injection of some innocuous substance, the animals will develop encephalitis and die. If, however, the virus is mixed with an immune serum, nearly all the mice will survive. This test has been used extensively for over one and a half years, and has proved to be very satisfactory (Hughes and Sawyer, 1932).

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The results obtained with the direct intracerebral test seemed to be sufficiently encouraging to justify further trials of this technique, with the object of devising a method for the quantitative study of the protective action of yellow fever immune serum. Such a method would have an advantage in that only small quantities of serum and virus would be required.

In this paper, the observed phenomenon that mice inoculated intracerebrally with virus and immune serum mixed in requisite proportions live, is termed 'protection,' without any intention of implying that the protective action occurs only *in vivo*. The evidence at present is insufficient to warrant any conclusion as to the mechanism of the reaction, i.e., whether there is neutralization which occurs *in vitro* or protective action which takes place only *in vivo*, or in a combination of both.

EXPERIMENTS WITH FRESH VIRUS

Success in devising a satisfactory intracerebral protection test would depend almost entirely upon developing a method for making a standard virus preparation, so that results obtained in one experiment would be comparable with those obtained in another.

By employing the technique originally used for the preparation of the virus suspension, the author obtained rather irregular results (Theiler, 1931). Though an immune serum always showed some degree of protection, the amount of protection obtained with any one serum varied when it was tested at different times. As the virus suspensions used were each prepared from only one or two infective mouse brains, the results were interpreted as indicating that the virus content of the different infective mouse brains varied. To determine this point it is obvious that a method of titrating the quantity of virus in any preparation is necessary.

Bauer and Mahaffy (1930) have shown that the virus of yellow fever deteriorates rapidly if diluted with saline solution. This deterioration can be prevented to a large extent by using a diluent consisting of saline or distilled water to which has been added 10 per cent. or more of normal serum. By the use of such a diluent, Bauer (1931) has titrated the virus content of infective monkey serum. In all the experiments reported here, unless otherwise stated, the diluent used for the preparation of virus suspensions, or for making dilutions of an immune serum, consisted of saline solution or distilled water containing 10 per cent. normal human or normal monkey serum.

To eliminate as far as possible the factor of differences in virus content of individual mouse brains, several virus preparations were made in which numerous infective mouse brains were used. The brains were always taken from mice that were obviously sick on the fourth or fifth day after an intracerebral injection of a virus fixed for mice. The brains were thoroughly ground up in a mortar, a measured quantity of diluent was added, and the suspension was centrifuged for half an hour at 2,500 revolutions a minute. From the supernatant fluid, tenfold dilutions were made, and 6 mice were inoculated intracerebrally with 0·03 c.c. of each dilution. Mice that died before the fourth day after inoculation were excluded from the series, since in our experience even the most potent yellow fever virus never kills before the fourth day. The mice that were found dead on the fourth to the tenth day inclusive were assumed to have died of yellow fever infection. Deaths due to a fixed virus after the tenth day are comparatively rare, even when the virus is tested in high dilution. The number of mice alive, therefore, on the tenth day after inoculation was taken as the number which survived.

The minimum lethal dose of a yellow fever virus fixed for mice was defined as the smallest quantity of virus which, when injected intracerebrally, would kill, on the average, half of the mice inoculated in 4 to 10 days after the inoculation. The minimum lethal dose is expressed in this paper, unless otherwise stated, as a dilution of infective mouse brain (without any regard for the virus lost by centrifugation), which, when injected intracerebrally in a volume of 0·03 c.c., fulfils this requirement. Furthermore, for the sake of simplicity, throughout this paper the titre of the virus will be stated in terms of the dilution of which 0·03 c.c. was the volume injected. The results of titrations of numerous virus preparations show that the minimum lethal dose of the preparations made according to a uniform technique varied from 1/100,000 to more than 1/2,000,000. The minimum lethal dose of most of the preparations was 1/200,000. Though the small number of mice (6) used for testing each dilution often made it difficult to determine accurately the minimum lethal dose, the method was considered sufficiently accurate to permit titration, on a roughly quantitative basis, of the protective action of an immune serum.

The results of such an experiment in the titration of serum are shown in Table I. The action of an immune serum in three concentrations, namely, undiluted, diluted 1/10, and diluted 1/100, was compared with the action of a normal human serum, when mixed with three dilutions of virus. The virus preparation was made from 11 mouse brains taken from mice definitely sick on the fifth day after inoculation with virus fixed for mice. The serum-virus preparation was injected soon after mixing. The dilutions of the immune serum were prepared with the same diluent used in making the virus dilutions. Consequently, in all the mixtures the concentration of normal serum was never less than 10 per cent. The results of this experiment (Table I) show that the protective action

TABLE I

Fate of mice inoculated intracerebrally with 0·03 c.c. of mixtures consisting of equal parts of various virus dilutions and three concentrations of an immune serum.

Virus dilution	Serum	Number of mice inoculated	Number of mice which died. Time in days after inoculation							Number of mice which survived
			4	5	6	7	8	9	10	
1/100	Undiluted immune	6	...	...	1	...	1	...	2	2
	1/10 immune	6	...	...	...	1	4	...	1	0
	1/100 immune	5	...	...	...	1	3	1	...	0
	Undiluted normal	6	...	...	3	2	1	...	...	0
1/1,000	Undiluted immune	6	...	...	...	...	...	...	...	6
	1/10 immune	6	...	1	...	...	...	1	1	3
	1/100 immune	6	...	...	...	1	1	...	...	4
	Undiluted normal	5	...	...	2	2	1	...	...	0
1/10,000	Undiluted immune	6	...	...	...	...	...	...	...	6
	1/10 immune	6	...	...	...	...	...	...	2	4
	1/100 immune	6	...	...	...	1	2	...	...	3
	Undiluted normal	6	...	...	...	3	3	...	...	0
1/100,000	Undiluted normal	6	...	...	1	...	3	...	...	2

of the immune serum could be demonstrated even when the serum was diluted one-hundredfold. Several additional experiments along the same lines gave similar results.

In these experiments the dilution of virus used was, as a rule, 1/10,000. When this dilution was mixed with an equal part of serum, the final dilution, as judged from various titrations, contained roughly from 10 to 100 minimum lethal doses in 0·03 c.c. of inoculum. As some protective action of a 1 in 100 dilution of each of several immune sera could be shown when this dilution of virus was used, the possibility of adapting the intracerebral method for the testing of unknown sera for immune bodies was investigated. Several sera were on hand from persons who had passed through attacks of yellow fever from 53 to 78 years previously, and who had not been exposed to yellow fever since the epidemic in Louisiana in 1905, 25 years before their sera were obtained. Five-tenths of a cubic centimetre of each serum was mixed with 0·5 c.c. of a 1/10,000 virus suspension, and every mixture was injected in 0·03 c.c. amounts into each of 6 mice. The results obtained are shown in Table II.

TABLE II

The protective action of six sera from persons who had yellow fever 53 and 78 years ago, against a 1/10,000 dilution of fresh virus.

Serum	Years between attack and taking of serum	Dilution of virus	Number of mice inoculated	Number of mice which died. Time in days after inoculation							Number of mice which survived
				4	5	6	7	8	9	10	
AJB	53	1/10,000	6	...	...	...	...	...	1	...	5
AL	53	1/10,000	6	...	...	...	...	...	1	...	5
HLJ	53	1/10,000	6	...	..	...	...	1	...	...	5
RER	53	1/10,000	6	...	...	...	...	...	...	...	6
MII	53	1/10,000	6	...	...	...	...	...	...	...	6
LGS	78	1/10,000	6	...	...	...	...	...	...	..	6
TN	Recent laboratory infection	1/10,000	6	...	...	...	...	...	1	...	5
LMM	Normal human	1/10,000	6	...	1	1	1	2	...	1	0
	Normal monkey	1/10,000	6	...	...	1	2	2	...	...	1
	Normal monkey	1/10,000	6	...	2	1	...	1	...	...	2

All the sera mentioned in this article from persons who had attacks of yellow fever 53 years ago or more, were investigated by Sawyer (1931) in a study of the persistence of yellow fever immunity. The initials identifying the sera in this article are the same as those used by Sawyer, who gives further details concerning the history of the donors. Study of Table II shows that all the sera tested gave definite protection.

In Table III are shown the results obtained when 3 sera from persons who had had yellow fever 53 years before were tested against a 1/1,000 dilution of virus—that is, 10 times the amount of virus used in the preceding experiment. These 3 sera, as well as one serum obtained from a person who had had a recent laboratory infection with yellow fever, were tested in 3 concentrations, namely, undiluted, and diluted 1/10 and 1/100. As controls, 11 mice were inoculated with a mixture consisting of equal parts of normal human serum and the same 1/1,000 dilution of virus. Two additional groups of mice were inoculated, one with 1/10,000 and one with a 1/100,000 dilution of virus. According to our definition, 0·03 c.c. of the

TABLE III

Fate of mice inoculated intracerebrally with 0·03 c.c. amounts of mixtures consisting of equal parts of 1/1,000 dilution of virus and three concentrations of immune sera.

Serum	History of serum	Dilution of serum	Dilution of virus	Number of mice inoculated	Number of mice which died. Time in days after inoculation							Number of mice which survived
					4	5	6	7	8	9	10	
AL	Yellow fever 53 years before bleeding	Undiluted	1/1,000	6	...	...	...	1	...	...	...	5
		1/10	1/1,000	6	...	...	...	...	...	...	...	6
		1/100	1/1,000	6	...	...	...	1	2	2	1	0
HLJ	Yellow fever 53 years before bleeding	Undiluted	1/1,000	5	...	...	...	...	...	...	...	5
		1/10	1/1,000	5	...	...	1	...	1	...	...	3
		1/100	1/1,000	6	...	...	...	2	...	1	...	3
RER	Yellow fever 53 years before bleeding	Undiluted	1/1,000	6	...	...	...	...	...	...	...	6
		1/10	1/1,000	6	...	...	...	...	2	...	...	4
		1/100	1/1,000	6	...	...	...	1	3	...	...	2
SFK	Recent laboratory infection	Undiluted	1/1,000	6	...	...	...	1	...	..	...	5
		1/10	1/1,000	6	...	...	...	...	...	...	...	6
		1/100	1/1,000	6	1	...	...	3	...	1	...	1
LMM	Normal	Undiluted	1/1,000	11	...	1	3	4	2	1	...	0
		...	1/10,000	6	...	...	...	2	3	1	...	0
		...	1/100,000	6	...	...	1	1	1	...	...	3

1/100,000 dilution is the minimum lethal dose of the virus used. The immune sera were, therefore, in this experiment, tested against 50 minimum lethal doses. All 4 sera showed definite protection, which was marked in every test.

EXPERIMENTS WITH FILTERED, FROZEN AND DRIED VIRUS PREPARATIONS

It became apparent during these tests that in virus suspensions, prepared according to the technique described above, the virus content varied too much for accurate work. The method of preparing the virus involved too many factors which were difficult to standardize. It was therefore decided to prepare the virus as usual, to filter it through a Berkefeld or Seitz filter, to distribute the filtrate in measured amounts in small test tubes, and to dry the virus while frozen, according to the technique which has been shown by Sawyer, Lloyd and Kitchen (1929) to be very satisfactory for the preservation of yellow fever virus. The test tubes containing the material so prepared were sealed and stored in a refrigerator at 4° to 6° C. At different times tubes were taken out of storage; sterile distilled water was added to make up to the original volume; various dilutions were prepared; and these dilutions were tested by intracerebral injection into mice. At first, tenfold dilutions were tested in order to determine roughly to what extent deterioration of the virus had occurred. Later, when it was found that deterioration usually took place slowly, twofold dilutions were adopted to determine more accurately the minimum lethal dose.

The results of a typical titration are shown in Table IV. According to the definition adopted, 0·03 c.c. of a 1/51,200 dilution is the minimum lethal dose. It is manifest that 6 mice for each dilution is entirely too small a number for the exact determination of the minimum lethal dose of a virus preparation.

By combining a large number of titrations such as the foregoing, Table V was compiled. In the enumeration of the mortality ratios, those groups in which there was one death or more before the fourth day are excluded. The last three columns of the Table show the total number of mice alive on the fourth day after inoculation, and

TABLE IV

Example of a titration of a preserved virus preparation

Dilution of virus	Number of mice inoculated	Number of mice which died. Time in days after inoculation										Number of mice which survived
		1	2	3	4	5	6	7	8	9	10	
1/800	6	...	...	...	...	...	1	3	2	...	...	0
1/1,600	6	...	...	...	...	...	2	...	3	...	...	1
1/3,200	6	...	...	...	...	1	1	1	2	...	...	1
1/6,400	6	...	...	...	...	...	...	1	2	...	...	3
1/12,800	6	1	...	...	...	...	...	...	2	...	...	3
1/25,600	6	...	...	...	...	...	1	...	...	1	...	4
1/51,200	6	...	...	...	...	...	1	2	1	...	...	2
1/102,400	6	...	...	...	...	..	1	...	...	...	...	5
1/204,800	6	. .	...	..	...	...	...	...	1	...	...	5

the number and percentage which died after injection with different amounts of virus. Although the definition of the minimum lethal dose is that amount of virus which will kill half the mice inoculated, it will be seen that as many as two-thirds of the mice died after injection with one minimum lethal dose. This becomes plain when it is seen that the mortality ratio was as high as 4/6 on 11 occasions and even 6/6 on 3 occasions. It is felt that Table V shows with a fair degree of accuracy the susceptibility of mice when inoculated with various amounts of virus.

The titration of various preserved preparations of virus has incidentally added considerably to our knowledge of the dependability of the method for the preservation of yellow fever virus. Several preparations had to be discarded for various reasons. In one, deterioration was so rapid that after 2 months the virus seemed to be totally inactive. In this preparation, the minimum lethal dose 6 days after preparation was 1/300,000; 14 days after preparation, it was 1/20,000; in 27 days, it was 1/1,000; and in 31 days, less than 1/100, the lowest dilution tested. Three preparations were of no use, because the virus became inactive in a number of tubes, although the virus content of some tubes was constant as far as was

TABLE V

Number of times various mortality ratios were obtained in mice inoculated intracerebrally with from ¼ to 64 minimum lethal doses, and the percentage of mice which died when inoculated with the same number of minimum lethal doses.

Number of minimum lethal doses	Number of tests	Number of times indicated mortality ratios were obtained. Numerator represents number of mice which died; denominator, number of mice inoculated							Total number of mice inoculated and alive on 4th day	Number of mice which died	Percentage of mice which died
		0/6	1/6	2/6	3/6	4/6	5/6	6/6			
64	12	0	0	0	0	0	1	11	75	73	97·3
32	54	0	0	0	0	0	13	41	350	335	95·7
16	26	0	0	0	1	1	5	19	157	146	93·0
8	24	0	0	0	2	3	10	9	178	155	87·1
4	32	0	0	2	3	8	14	5	193	147	76·2
2	29	0	3	3	4	11	5	3	171	113	66·1
1	26	...	...	...	11	11	1	3	168	112	66·7
½	23	2	8	13	...	...	...	..	148	41	27·7
¼	30	10	12	8	...	...	...	...	111	19	17·1

determined by a few titrations. Even in some of the best preparations, virus could not be demonstrated in a few of the tubes. In view of this, it was considered advisable to mix the contents of two tubes whenever a protection test or titration was to be done.

In making the virus preparations, the drying was carried out in two or more desiccators. A variable was thus added to different lots of the same virus preparation; and, although numerous titrations have shown that, as a rule, the activity of the end products of the different desiccators is the same, this is, nevertheless, a serious disadvantage in the method, as the virus from each desiccator must be considered as a different product until shown by several titrations to be identical. As a rule, 12 tubes were dried in each desiccator; and, as 2 tubes are used for each titration, it is evident that the contents of one desiccator cannot be used for a large number of experiments.

RATE OF DETERIORATION OF PRESERVED VIRUS PREPARATIONS

In Table VI are listed the end points of representative titrations performed at different intervals after the manufacture of the first 3 virus preparations. It will be seen that with each preparation there was a considerable period of time when the end point was fairly constant. It will also be observed that the rate of deterioration was not the same for the different preparations. Thus the end point for preparation 1, which after 24 days' storage had a minimum lethal dose between 1/20,000 and 1/200,000, fell to 1/800 after 389 days. Preparation 3 remained remarkably constant for 259 days, when the end point varied from 1/40,000 to 1/160,000. After 394 days' storage, the end point was 1/20,000.

In addition to the time variable, there are two other variables in the above-mentioned titrations, namely, the strains of mice, and the diluents. As will be shown later, the strains of mice used made no appreciable difference. This cannot be said for the diluent. Evidence will be presented that different normal sera vary considerably in their usefulness as diluents for yellow fever virus. Furthermore, only 6 mice were used for testing each dilution of virus, and consequently, on account of the great range of susceptibility of different individuals of the same strain of mice, the end point was very seldom clear cut. It was felt, and further experience amply bore this out, that the virus content of the different tubes did not vary as much as one would be led to believe by the results of the titrations recorded in Table VI.

STRAIN OF MICE

Sawyer and Lloyd (1931) made the important discovery that different strains of mice varied in their susceptibility to the virus of yellow fever. In quantitative work it is obvious, therefore, that the degree of susceptibility should be determined as accurately as possible. In Table VII are recorded the results of titrations of the same virus preparation in 4 different strains of mice. The Institute strain of mice appears much more resistant than the other three strains. The 3 susceptible strains, namely, the Dilute Brown, the B. and the Swiss strains seem equally susceptible. A finding not

TABLE VI

Results of titrations of three preserved virus preparations after various intervals of storage.

Number of preparation	Age, in days, of preparation	Highest dilution which, when injected intracerebrally in 0·03 c.c. amounts, killed half the mice from 4th to 10th day after injection
1	0	1/200,000
	24	Between 1/20,000 and 1/200,000
	123	1/6,400
	175	1/25,600
	185	1/51,200
	389	1/800
2	190	1/6,400
	224	1/12,800
	231	1/25,600
	242	1/25,600
	243	1/51,200
	260	1/25,600
	335	1/6,400
	356	1/6,400
	419	1/12,800
3	92	1/160,000
	137	1/80,000
	160	1/160,000
	192	1/80,000
	220	1/40,000
	258	1/160,000
	259	1/160,000
	394	1/20,000

TABLE VII

The mortality of four strains of mice after intracerebral injections of 0·03 c.c. of different dilutions of the same virus preparations

Dilution of virus	Institute strain		Dilute Brown strain		B. strain		Swiss strain	
	Number inoculated	Number died 4-10 days	Number inoculated	Number died 4-10 days	Number inoculated	Number died 4-10 days	Number inoculated	Number died 4-10 days
1/800	6	1	6	6	11	9	12	11
1/1,600	6	2	6	6	12	11	12	12
1/3,200	6	4	6	5	12	10	12	12
1/6,400	6	1	6	5	11	9	11	10
1/12,800	6	3	6	4	11	7	12	7
1/25,600	6	0	6	3	12	7	11	5
1/51,200	6	2	6	2	11	6	12	7
1/102,400	5	0	6	2	11	3	11	4
1/204,800	6	0	6	1	10	1	12	2
Total ...	53	13	54	34	101	63	105	70
Percentage of mice which died	24·5		63·0		62·4		66·7	

shown in the Table was that the time of death in the Dilute Brown strain was far more regular than in the other three. With this strain, all deaths occurred from the fifth to the eighth day after inoculation, and the great majority of the mice that succumbed died on the sixth and seventh days. For practical purposes, the susceptibility of the Swiss, Dilute Brown and B. strains were considered equal. The Swiss and B. strains were chiefly used in the work recorded in this article.

INFLUENCE OF AGE ON THE SUSCEPTIBILITY OF MICE

Age and diet have often been held to have an influence on the susceptibility of a host to a given infectious agent. Theiler (1930) has shown that suckling mice are much more susceptible than adult mice to an intradermal or subcutaneous injection of yellow fever virus. It seemed, therefore, important to determine whether age is a factor in the susceptibility of mice to an intracerebral injection of virus. Mice of the Swiss strain, raised on the same diet, were divided into two lots according to age, as indicated by weight. The individuals of the one lot weighed less than 15 grams.; those of the other, more than 18·5 grams. Six mice of each lot were inoculated with dilutions of the same virus preparation. The results are shown in Table VIII. No difference in susceptibility is manifest between the two lots. In both, the minimum lethal dose of the virus preparation was 1/51,200. The total number of mice that died was also the same in the two lots. From these results it may be concluded that age, within the limits tested, has no significant influence on the susceptibility of mice to yellow fever virus.

TABLE VIII

The influence of age on the susceptibility of mice to intracerebral injections of virus

Dilution of virus	Young mice, 15 grams or less		Old mice, 18·5 grams or more	
	Number of mice inoculated	Number of mice which died 4-10 days	Number of mice inoculated	Number of mice which died 4-10 days
1/800	6	6	6	5
1/1,600	6	6	6	6
1/3,200	6	6	6	6
1/6,400	5	4	6	6
1/12,800	6	5	6	2
1/25,600	6	1	5	4
1/51,200	6	4	6	3
1/102,400	5	2	6	2
1/204,800	6	1	6	1
Total ...	52	35	53	35

INFLUENCE OF DIET ON THE SUSCEPTIBILITY OF MICE

In Table IX are recorded the results obtained by the injection of various dilutions of one virus preparation into two lots of mice of the Swiss strain. We are indebted to Dr. Alexis Carrel, of the

TABLE IX

The influence of diet on the susceptibility of mice to an intracerebral injection of virus.

Dilution of virus	Mice raised on a deficient diet		Mice raised on an adequate diet	
	Number of mice inoculated	Number of mice which died 4-10 days	Number of mice inoculated	Number of mice which died 4-10 days
1/400	5	5	3	3
1/800	4	4	6	6
1/1,600	5	3	5	4
1/3,200	5	5	5	4
1/6,400	5	3	4	3
1/12,800	5	2	5	2
1/25,600	6	2	6	2
1/512,000	6	1	6	2
1/102,400	6	1	6	0
1/204,800	6	1	6	0
Total ..	53	27	52	26

Rockefeller Institute for Medical Research, for the interest and co-operation in supplying all the mice used in this experiment. One lot represented the third generation of a series of mice that had been raised on a deficient diet consisting principally of meat, potatoes and bread, with a little cabbage and lettuce. The other lot was raised on an adequate diet. The individuals of the first lot were small and had ruffled fur, whereas those of the second were large, sleek and healthy. The Table shows quite clearly that even such extremes of diet had no significant influence on the susceptibility of the mice to intracerebral injection of virus.

THE DILUENT

After it had been shown by Bauer and Mahaffy (1930) that the virus of yellow fever deteriorates rapidly in physiological salt solution, and that this deterioration could to a large extent be prevented by the addition of 10 per cent. or more of normal human or normal monkey serum, most workers have adopted some diluent containing normal serum. As soon as it became evident that normal sera differed in their suitability for use in diluents, search was made for some suitable substance which could be obtained in large quantities, so that the same lot of diluent could be used over an extended period of time. At the suggestion of Dr. J. H. Bauer, different specimens of human ascitic and pleuritic fluids were tested for their suitability. Dilutions of virus were made in the fluid to be tested, and these were injected into mice. For control, similar dilutions of the same virus were made in a diluent whose suitability was known. In order to bring out more markedly any differences which might exist, the dilutions of virus were usually incubated at 37° C. for 24 hours before injection. In this way, several satisfactory diluents were obtained in large quantities.

The optimum concentration of normal serum in a diluent has not been determined. As a routine, 10 per cent. was adopted. In one instance, however, in which the same virus was titrated in two diluents, one containing 10 per cent. and the other 55 per cent. of the same serum, many more mice died among those that were inoculated with the dilutions of virus made up in 55 per cent. serum. This seemed to indicate that the more concentrated diluent was the more suitable. In another experiment, exactly opposite results were obtained: the diluent containing 10 per cent. of a normal serum proved more suitable than the more concentrated diluent. Hence, at present very little information is available as to the most suitable concentration of normal serum to be used as a diluent.

The availability of large quantities of ascitic and pleuritic fluids simplified work immensely, and added to its accuracy. The great disadvantage was that every new fluid had thoroughly to be tested before being introduced. At the suggestion of Dr. T. P. Hughes, it was decided to test various concentrations of egg albumin. A 2·5 per cent. solution of egg albumin in distilled water, sterilized by filtration, was found to be satisfactory; it proved to be better, in fact, than an ascitic fluid with which it was compared and which had been found very satisfactory. But, as the egg albumin diluent has not been in use for long, it is impossible at present to give a final opinion as to its efficiency.

PROTECTION TESTS WITH FROZEN AND DRIED VIRUS

In comparison with the preparations of virus freshly made each time, titrations of the product dried in the frozen state gives a much more constant end point. In protection tests, it is apparent that the amount of virus used is important. It is conceivable that certain yellow fever sera would be so weak that no protection would be demonstrated if they were tested against an excessive amount of virus. Furthermore, in view of the great difference in susceptibility of individual mice of the same strain, a dose of virus should be selected which will kill nearly all mice with great regularity. A dose which would kill all mice would be unsuitable, since in all the strains of mice tested so far there is always a certain small percentage resistant to many lethal doses.

Numerous protection tests have been carried out in which an immune serum or dilutions of an immune serum have been tested against 4 to 32 minimum lethal doses of virus. In Table X are

TABLE X

The protective action of immune sera when tested against thirty-two minimum lethal doses.

Serum	History of donor	Dilution of serum	Number of mice which died. Time in days after inoculation								Number of mice which survived
			1-3	4	5	6	7	8	9	10	
A.J.B.	Yellow fever 53 years ago	Undiluted	...	...	...	...	...	..	...	...	6
E.P.L.	Yellow fever 53 years ago	Undiluted	...	...	...	...	...	...	...	...	6
E.L.R.	Yellow fever 53 years ago	Undiluted	...	...	...	...	...	...	...	...	6
R.W.I.	Yellow fever 64 years ago	Undiluted	...	...	...	...	...	...	...	...	6
J.M.	Yellow fever in Rio in 1928	Undiluted	...	...	...	...	...	...	...	...	6
		1/10	1	...	...	1	...	...	...	...	4
		1/100	...	...	...	...	...	...	...	...	6
M.A.	Yellow fever in Rio in 1928	Undiluted	...	...	...	...	...	...	...	...	6
		1/10	...	...	...	...	...	...	...	...	6
		1/100	...	...	...	1	...	1	...	...	4
Immune Monkey		Undiluted	...	...	...	...	...	...	...	...	6
		1/10	...	...	...	...	...	...	...	...	6
		1/100	...	...	...	...	...	...	...	...	6
M.T.	Recent laboratory infection	Undiluted	...	...	...	...	...	...	...	...	6
		1/10	...	...	...	...	...	...	...	...	6
		1/100	...	...	...	...	...	2	1	...	3
L.M.M.	Normal human	Undiluted	...	...	...	1	2	3	...	...	0
		Undiluted	...	...	1	1	1	3	...	...	0

shown the results of such a test. The action of seven human immune sera and one monkey immune serum was tested against 32 minimum lethal doses. Four of these sera were from persons who had passed through an attack of yellow fever 53 to 64 years ago. Four sera were tested in 3 concentrations, namely, undiluted, diluted 1/10, and diluted 1/100. Equal parts of the serum or serum dilution were mixed with the virus dilution, and after these mixtures had been allowed to stand at room temperature for several minutes, they were injected in 0·03 c.c. amounts intracerebrally into mice. Six mice were used for each mixture. Two groups of 6 mice each were inoculated with a mixture of normal serum and virus. All the controls died, whereas nearly all the mice that received the mixtures of immune serum and virus lived.

The duration of immunity to yellow fever is said to be lifelong. Whether or not immunity is always accompanied by demonstrable antibodies in the serum is unknown. Sawyer (1931) made an extensive study of the persistence of yellow fever immunity by means of the protection test in monkeys, supplemented in a few instances by intraperitoneal protection tests in mice. From his results he concluded that the concentration of antibodies in the serum may in some cases gradually diminish until they are no longer demonstrable by protection tests with ordinary amounts of the serum. These conclusions are based almost entirely on protection tests in monkeys, but are nevertheless suggestive. A test, therefore, for the demonstration of antibodies should be standardized, if possible, so that even the weakest sera of persons who have had yellow fever will show protection. Consequently, a study was undertaken to determine the concentration of antibodies in the sera of persons who had had the disease many years before. There were available 9 sera of persons who had had yellow fever 53 or more years before, and who had not been exposed to the disease during the 25 years prior to the time when their sera were obtained. All but 2 of the sera were titrated, most of them 2 or 3 times. Table XI gives a summary of the results obtained. The number of minimum lethal doses against which the sera or dilutions were tested, i.e., the number of minimum lethal doses in each inoculum, varied from 4 to 32. It will be observed that every serum protected at least 5 out of 6 mice, when tested undiluted. An intracerebral

TABLE XI

Results of titrations of sera from persons who had yellow fever 53 to 64 years before bleeding.

Serum	Years between attack and taking of serum	Protection test in *Macacus rhesus* monkeys; result in test animal; days to fever or death (after Sawyer 1931)	Results of protection tests in mice: Protective serum: Highest dilution of serum which protected 5 or 6 of 6 mice injected	Results of protection tests in mice: Protective serum: Highest dilution of serum which protected 3 or 4 of 6 mice injected	Results of protection tests in mice: Dose of virus: Number of minimum lethal doses	Results of protection tests in mice: Normal serum controls: Number of mice inoculated	Results of protection tests in mice: Normal serum controls: Number of mice which died	Remarks
A.J.B.	53	1½ days to fever; survived	Undiluted		32	12	12	Serum tested only undiluted
A.L.	53		Undiluted	1/10	16	24	24	
E.P.L.	53	Remained well	Undiluted		32	12	12	Serum tested only undiluted
H.L.J.	53	1½ days to fever; 5½ to death	1/2	1/8	32	24	24	
E.L.R.	53		1/100		4	23	19	1/200 dilution allowed 2 of 6 mice to live
R.W.I.	64	Remained well	1/16		32	24	24	1/32, 1/64 and 1/128 dilutions allowed 2 of 6 mice to live
R.E.R.	53	Remained well	1/4	1/64	32	24	24	
M.H.	53	Remained well	1/10	1/50	32	24	22	
C.O.T.	53	1½ days to fever; 7 to death	1/10		16	24	24	

protection test, standardized so that each mouse received 32 minimum lethal doses, would seem, therefore, to be satisfactory. With this concentration of virus, definite protection has been shown with every known immune serum so far tested. Furthermore, this concentration of virus kills nearly all the control mice with great regularity. Of 120 mice receiving 32 minimum lethal doses in normal serum (Table XI), 118 died from the fourth to the tenth day inclusive. The results in these cases are therefore very clear cut.

By using the small number of 6 mice for testing each dilution, only a very rough idea of the potency of a serum can be obtained. It is suggested that for accurate work at least 12 mice be used, and that the highest dilution of serum which will on the average protect half the number of mice inoculated be taken as the end point.

The highest dilution of the sera of persons who had recently had yellow fever, which protected 5 or 6 mice against 32 minimum lethal doses of virus, was usually 1/100 or 1/200. When these sera were tested against a smaller amount of virus, the titre was much higher. In using the methods outlined above for determining the titre of an immune serum, it is essential to state in every case the number of minimum lethal doses employed.

SUMMARY

The possibility of a simple intracerebral test in mice for determining quantitatively the amount of protective antibodies in yellow fever immune serum was investigated. The method consisted of mixing the virus suspension with an equal amount of the serum or serum dilution under investigation, and injecting the mixture in 0·03 c.c. amounts intracerebrally into 6 or more mice of a susceptible strain. Two methods of preparing the virus were investigated. In the first, the virus was prepared fresh each time from a number of infective mouse brains, taken when the mice were obviously sick on the fourth or fifth day after an intracerebral injection of passage virus. The infective mouse brains were ground in a mortar, diluent was added, and the suspension was centrifuged at 2,500 revolutions per minute for 30 minutes. From the supernatant fluid, tenfold dilutions were made and injected into mice to determine the minimum lethal dose. Numerous titrations of virus preparations made according to this standardized method showed that the minimum lethal dose varied from 1/100,000 to 1/2,000,000. By using a 1/10,000 dilution of virus, the protective action of numerous sera from persons who had recently had yellow fever was shown. All of 6 sera from persons who had had yellow fever 53 years before bleeding showed definite protection.

On account of the great range in the minimum lethal dose of the virus suspensions prepared fresh each time, the possibility of using preserved virus preparations was investigated. Suspensions of infective mouse brains were prepared, filtered through Berkefeld N or Seitz filters, dried in measured quantities while frozen, according to the technique of Sawyer, Lloyd and Kitchen, and stored in the refrigerator at 4° to 6° C. At various intervals extending over one year, the minimum lethal dose of the preserved preparations was determined. By this means it was shown that the rate of deterioration of the virus preparations, although these were made in accordance with a uniform technique, varied considerably. In approximately half the preparations, the end point of the titration was so constant over a long period of time that the preparation could be used repeatedly and the results of one experiment could be compared with those of another.

By the use of preserved virus preparations it was shown that age and diet, within the limits investigated, had no influence on the susceptibility of the mice to an intracerebral injection of virus.

The finding of Sawyer and Lloyd that different strains of mice show various degrees of susceptibility to the virus of yellow fever was confirmed. It was also shown that each of the different strains of mice used in these experiments exhibited a relatively constant susceptibility.

By mixing the serum under investigation with a suspension of virus so diluted that when it was injected in 0·03 c.c. amounts intracerebrally each mouse received 32 minimum lethal doses, the protective action of numerous yellow fever sera, including 7 from persons who had had yellow fever 53 or more years before, was shown. In every case, all or all but one of 6 mice inoculated were alive on the tenth day after inoculation. Of 120 control mice that received mixtures of normal sera and the same virus suspension, only 2 were alive 10 days after inoculation.

For accurate work, on account of the great range of susceptibility of mice of a given strain, it is suggested that 12 mice should be used for each dilution of virus or serum in titration experiments.

The limitations of the method of preserving virus by drying it in the frozen state makes the procedure unsuitable for use in the routine testing of sera by the method here presented. And before means are found for preserving the virus in large amounts, so that the same preparation can be used repeatedly over a large interval of time, it would be premature to advocate the method for the routine testing of yellow fever sera in epidemiological work.

During the course of this work, the great importance of the diluent became manifest. Yellow fever virus deteriorates rapidly in 0·9 per cent. sodium chloride solution. The addition of 10 per cent. or more of normal serum prevents this. It was found that different normal sera varied considerably in their suitability for use in diluents. To overcome this, ascitic and pleuritic fluids which were obtained in large quantities were investigated, and several were found to be quite satisfactory. It was also found that a 2·5 per cent. solution of egg albumin in distilled water could be used as a diluent.

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A NEW SPECIES OF *CULICOIDES* FROM PALESTINE

BY

J. W. S. MACFIE

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I am indebted to Dr. P. A. Buxton, London School of Hygiene and Tropical Medicine, for the opportunity of examining and describing the following insect.

CULICOIDES JUDAEAE sp. nov.

A small greenish-brown species, with unadorned thorax and wings.

MALE, FEMALE. Length of wing, about 1 mm.; greatest breadth of wing, 0·4 mm. or less.

Head dark brown. Palpi brown, third segment inflated (especially in female) and furnished with a large, shallow pit in which are massed numerous short sensory organs; lengths of last 3 segments in one female 20, 8 and 8 units respectively. Antennae brown, much paler in male than in female. In male, last 3 segments elongate, their lengths in the single specimen examined 27, 20 and 22 units respectively; the combined lengths of segments 4-12 and 13-15 approximately equal. In female, segments 4-10 oval to somewhat vase-shaped, in one specimen measuring from 8 by 6-7 to 8 by 5 units; 11-14 elongate, sub-equal, about 13-14 by 5 units; 15 longer, about 18 by 5 units, tapering distally, without stylet. The combined lengths of segments 11-15, 4-10, and 3-10 in this specimen, 73, 57 and 68 units respectively.

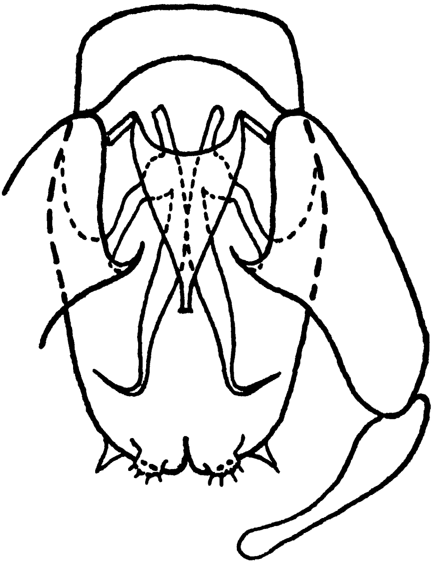
Thorax uniformly darkish brown, with a greenish tint. Scutellum similarly coloured; bearing 4 bristles and several smaller hairs (about 14 in female, 8 in male). Postscutellum darker brown.

Wings unadorned, slightly milky; well but not densely clothed with macrotrichia (not darkened over radial cells), which cover the entire surface with exception of radial and basal areas, and extend in female practically to base between M and Cu. Costa reaching a little beyond middle of wing. First radial cell slit-like, second small but well formed. End of third vein curved up towards costa at an obtuse angle. Petiole of M longer than cross-vein. Fork of Cu distal to that of M. Halteres with pale, brownish knobs.

Legs rather pale brown, with knees and apices of hind tibiae darker; paler in male than in female. Segments, claws and empodium normal. T.R. rather less than 2.

Abdomen darkish brown in dried insects. Spermathecae 2, rather poorly chitinised, spherical, sub-equal, about 55-60μ in diameter; the duct not at all chitinised. Hypopygium (see Fig.) darker brown. Posterior margin of ninth tergite with median cleft, and bearing a pair of rather short processes. Ninth sternite widely but not very deeply excavated. Harpes highly chitinised, with characteristic basal extensions, and distal extremities tapering to fine points which are bent ventrally. Aedeagus dark brown, triangular, tapering distally. Membrane joining ninth sternite to aedeagus not spiculate.

Palestine: 'Collected in the grounds of the Palestine Potash Company on the shores of the Dead Sea,' 1932, 1 ♂, 11 ♀♀.



Ventral view of hypopygium of *Culicoides judaeae*.

This is a much smaller and browner insect than either of the species from Palestine with unadorned wings described by Austen (1921), namely, *C. vitreipennis* and *C. puripennis*. It resembles most closely the European *C. pumilus* (Winn., 1852), a species which Edwards (1926) regards as the same as *C. minutissimus* (Zett., 1855). Thanks to the kindness of Dr. Edwards, I have been able to examine a British male *C. minutissimus* and to compare it with the Palestine species described above. The two are not identical, and differ notably in the form of the hypopygium. In *C. minutissimus* the ninth sternite is more deeply excavated than in the Palestine species, the lateral processes on the posterior margin of the ninth tergite are much longer, the harpes have long, twisted, filiform ends, and the aedeagus is more Y-shaped.

It should perhaps be noted here that in Winnertz's figure of the wing of *C. pumilus* the fork of Cu is shown slightly proximal to that of M. In his final key to the European species of *Culicoides*, Kieffer (1925) makes use of this character for the differentiation of the species, although he reproduces a figure of the wing (that of Goetghebuer) in which the reverse relationship is shown, namely, the fork of Cu slightly distal to that of M. As it is often difficult to see the exact point at which M forks without the aid of staining reagents, it would probably be unwise to attach great importance to this detail in Winnertz's figure. In the male *C. minutissimus*, kindly lent for examination by Dr. Edwards, the forking of Cu is clearly distal to that of M.

ON *ANOPHELES FUNESTUS* AND ITS ALLIES IN THE TRANSVAAL

BY

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(Received for publication 10 *January,* 1933)

The principle of 'species sanitation' depends for its success on the accurate identification of the insects concerned with the transmission of malaria. Unfortunately, in South Africa one of the main vectors of malaria, namely *A. funestus*, is a difficult insect to name with certainty both in adult and larval stages. Moreover, the recent discovery of another closely related form has complicated matters still further. It has therefore been thought advisable to publish these notes in an attempt to make the present position clear.

A. funestus belongs to the group *Myzomyia* of the subgenus *Myzomyia*, and its nearest allies are *A. marshalli* Theo., *A. longipalpis* Theo., *A. transvaalensis* Carter,* *A. rhodesiensis* Theo. and the recently discovered *A. funestus* var. *leesoni* Evans.† The author (1931) has already shown that in the larval stages *A. transvaalensis* and *A. marshalli* present no difficulty, as they are immediately distinguished by the branching of the anterior dorsal pleural hair of the mesothorax. *A. longipalpis* is known by the relatively small anterior abdominal plaques and by the fact that the posterior abdominal plaques, at least on the posterior segments, are three in number. The larva of *A. rhodesiensis* is our only *Myzomyia* with the inner clypeals barbed. The larva of *A. marshalli* further differs from all our *Myzomyia* larvae in having the saddle hair much branched (fig. 1, *a* and *b*).

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* It may be necessary to rename this anopheline, and attention is directed to a forthcoming paper dealing further with the species.—Edd.

† Evans (1931) described this as *A. funestus* subspecies *leesoni*.—Edd.

A. funestus var. *leesoni* was described by Evans (1931) in November, 1931, from material collected in Rhodesia. At about the same time this form was discovered in Tzaneen, and the following is an extract from a report to the Director of the South African Institute for Medical Research, dated 20th December, 1931 : ' I have now confirmed the observation set out in the monthly report for November, namely, that there exists another mosquito formerly confused with *funestus* but really quite distinct from it. Originally separable in the larval stage only it has now been found that the adult males are also distinguishable; the adult females remain inseparable. The absence of males from habitations indicates that this species does not enter houses. It was thought that this observation would clear up the whole problem of *funestus* with and without malaria, but it is now becoming evident that this is not the whole truth. Intensive collecting has shown that apparently true *funestus* larvae do occur in non-malarious areas. It is possible that a third species is involved but I am inclined to think that some ecological factor will be found to be the cause.'

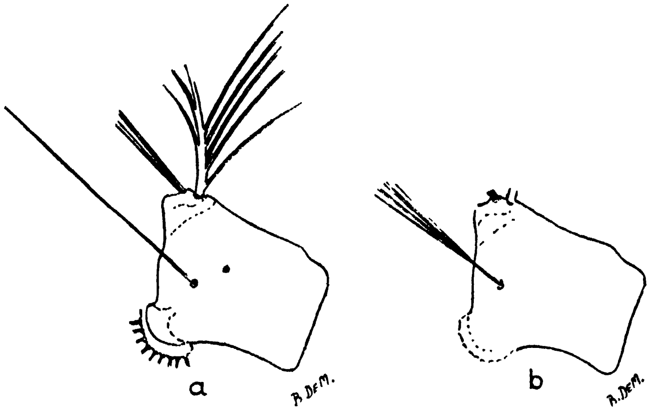


FIG. 1. *a.*—*A. funestus*, anal segment of larva showing lateral hair; *b.*—*A. marshalli*, anal segment of larva showing lateral hair.

In the larva of *A. funestus* var. *leesoni*, the long mesothoracic pleural hairs are both simple. It therefore falls into the same category as the larvae of *marshalli*, *longipalpis* and *funestus*. From these it is distinguished in the first place by the much branched inner occipital hair (fig. 2, *c*, *d* and *e*). From the larvae of *marshalli*

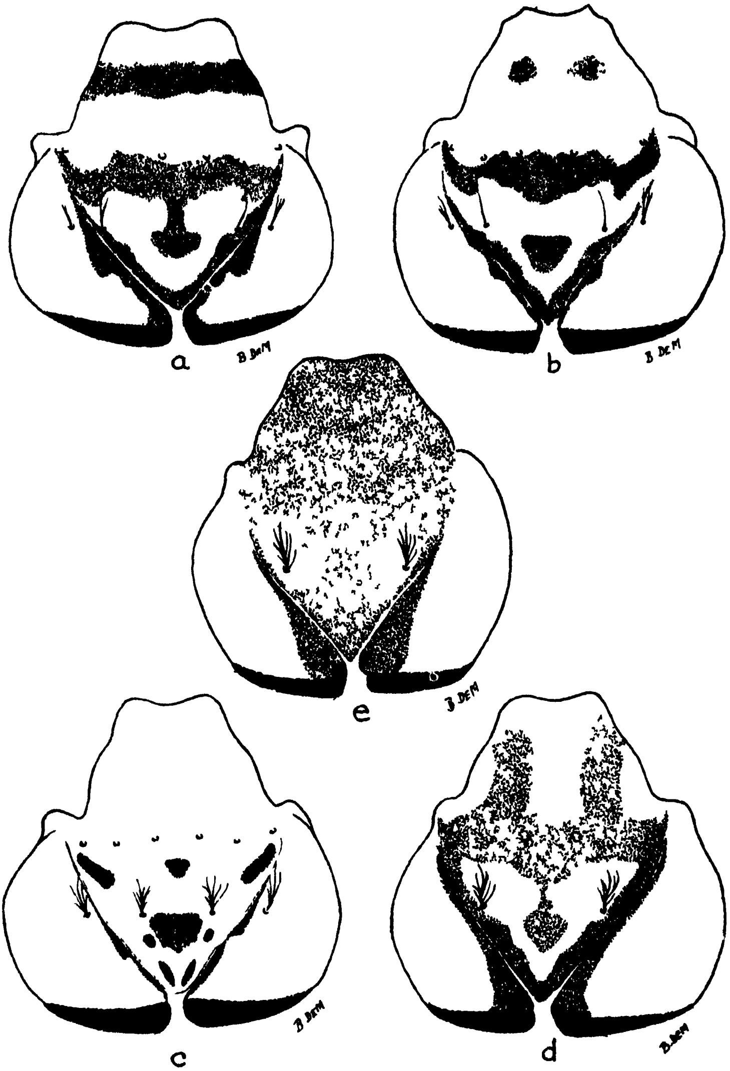


Fig. 2 *a*—*A funestus*, head of larva showing typical pigmentation, *b*—*A funestus*, head of larva showing atypical pigmentation, *c*—*A funestus* var *leesoni*, head of larva showing atypical pigmentation, *d*—*A funestus* var *leesoni*, head of larva showing typical pigmentation, *e*—*A. funestus* var. *leesoni*, head of larva showing atypical pigmentation and *longipalpis* one can recognise it by the relatively larger anterior abdominal plaques. It differs from *funestus* in the following respects :—

1. Inner occipital with 7-8 branches. We have seen a *funestus* larva in which one inner occipital had four branches. Nothing approaching the condition seen in var. *leesoni*, however, has ever before been noted.
2. Anterior head pattern consisting of two longitudinal stripes (fig. 2, *d*) instead of a transverse band (fig. 2, *a*). We have seen *funestus* in which the transverse band is not complete. The band is then represented by two small darkened areas, but one can usually trace a tendency for these two to unite transversely (fig. 2, *b*). Occasionally the anterior portion of the clypeus is quite unpigmented. Sometimes, in the larva of *funestus* var. *leesoni*, the two longitudinal stripes are completely absent (fig. 2, *c*), and we have seen one larva of this variety in which the whole clypeus, with the exception of two small areas at the bases of the inner occipitals, was pigmented. (fig. 2, *e*).

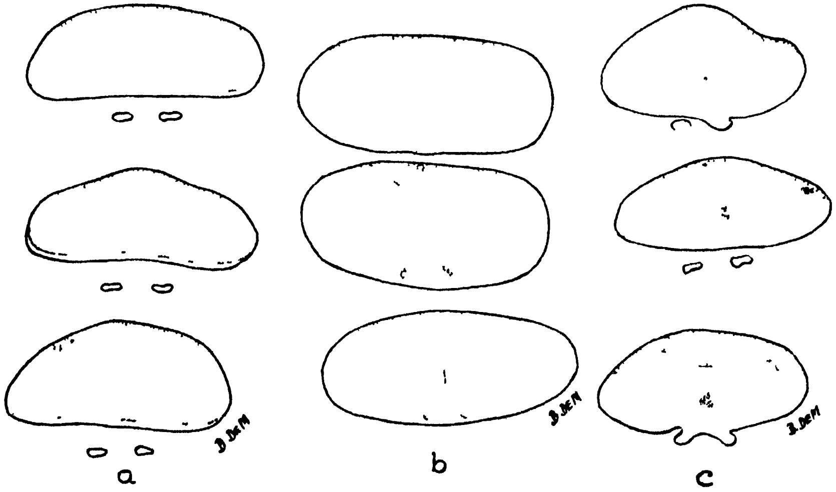


FIG. 3. *a.—A. funestus* var. *leesoni*, abdominal plaques V, VI and VII of the larva; *b.—A. funestus*, abdominal plaques V, VI and VII of the larva; with posterior plaques included in anterior plaques; *c.—A. funestus*, abdominal plaques V, VI and VII of the larva; with posterior plaques in the process of being included by the anterior plaques.

3. Two posterior plaques always present on some abdominal segments (fig. 3, *a*). Typical *funestus* has no posterior plaques (fig. 3, *b*). Sometimes, however, these are present

on the anterior abdominal segments and, rarely, on all segments. Usually, in *funestus*, posterior plaques can be distinguished in the young larva, or the process of their inclusion by the anterior plaques can be seen (fig. 3, *c*). When no posterior plaques are present in *funestus*, they can often, but not always, be seen as darkened areas lying in the anterior plaque (fig. 3, *b*). A scheme for separating these closely allied larvae is given as follows :—

1.	Anterior dorsal mesopleural hair, feathered	2
	Anterior dorsal mesopleural hair, simple	3
2.	Inner clypeals, simple	*transvaalensis*
	Inner clypeals, barbed	*rhodesiensis*
3.	Anterior plaque of abdominal segment VII more or less half as deep as the segment itself. This plaque about as wide as the segment	4
	Anterior plaque of abdominal segment VII much shallower, about one-fifth as deep as the segment. Plaque much narrower than the segment	5
4.	Inner occipital much branched. Anterior portion of the head with two longitudinal pigmented areas. Posterior plaques always present on some segments at least	*funestus* var. *leesoni*
	Inner occipital simple, or with at most four branches. Anterior transverse pigmented band usually present on the head. Posterior plaques usually absent ; occasionally present on anterior segments, rarely on all segments	*funestus*
5.	Filaments of abdominal palmate hairs short. One posterior plaque present. Saddle hair much branched. Larva pale in colour	*marshalli*
	Filaments long. Three posterior plaques present on some segments at least. Larva very dark in colour	*longipalpis*

The ventral surface of abdominal segments IV, V, VI, VII and VIII, but more especially segments VI and VII in the larva of *funestus*, is clothed with a large number of closely-set, sharp-pointed spines, with their apices directed towards the tail end of the larva (fig. 4). These possibly aid the larva in keeping a hold of the side

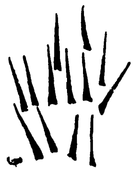


FIG. 4. *A. funestus*, spines from the ventral surface of abdominal segment VI of the larva. (× 600 approx.)

of the stream. It is a peculiar fact, however, that these spines are absent from the larva of *funestus* var. *leesoni*, although its breeding habits are very similar to those of *funestus*, and the two occur side by side in the same streams. Indeed, the larva of *A. theileri* is the only other stream breeder in which these spines are developed to any extent.

As pointed out by Evans in her original description, the pupae of *funestus* var. *leesoni* and *funestus* are easily separable. Some of the main differences are shown in figs. 5 and 6. It will be seen that bristle 'A' of segments V, VI and VII is relatively much longer in *funestus* var. *leesoni*; similarly, bristle 'B' is longer, and, furthermore, is simple in these segments, whereas in *funestus* it is bifid or triple.

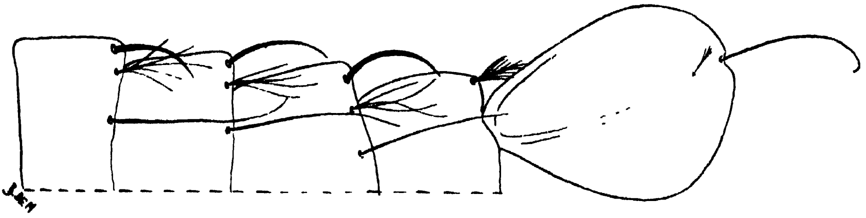


FIG. 5. *A. funestus* var. *leesoni*, portion of the pupa.

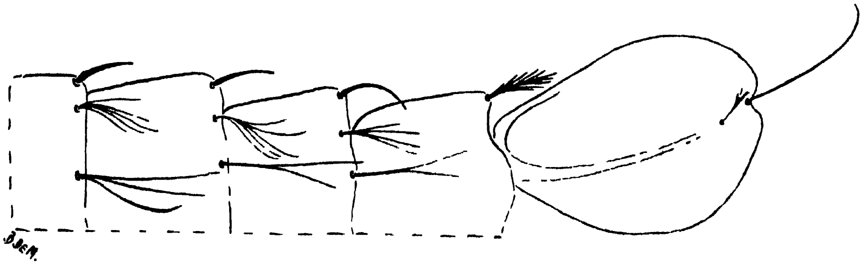


FIG. 6. *A. funestus*, portion of the pupa.

Breeding Places. A. marshalli and *A. transvaalensis* are typical seepage breeders in shaded water. They also occur to some extent along the edges of streams. *A. rhodesiensis* breeds usually in exposed pools and sometimes in rock pools. *A. funestus* and its variety occur side by side along the edges of shaded streams, less often in marshy ground where there is some movement of the water, and occasionally in seepages. It has repeatedly been pointed out that in South Africa *A. funestus* is not a marsh breeder. This has led to some misunderstanding because of the various ways in which the term ' marsh ' can be interpreted. By ' marsh ' we mean a collection of surface water, usually in the form of a number of small pools which are overgrown with vegetation and in which there is no movement of water. Very often it happens that a marsh or swamp has a central stream of slow moving water, as occurs in the Northern Rhodesian ' dhambo.' In such a case, larvae of *funestus* will, of course, be found, and the breeding place is no longer a marsh or swamp according to our terminology.

SEASONAL PREVALENCE OF *A. FUNESTUS* AND ITS VARIETY IN THE DRAKENSBERG FOOTHILLS

At Tzaneen, the larvae and adults of *funestus* and *funestus* var. *leesoni* both disappear in winter, whereas at Mohlaba, 12 miles distant, they breed all the year round. This is clearly shown by Graph I and Table I. Weekly collections of larvae in all types of breeding places were made at Tzaneen and Mohlaba and their environs during 11 months. Monthly totals were then made, and the percentage calculated of *funestus* and *funestus* var. *leesoni* of the total of all larvae.

It will be seen that *funestus* and its variety disappear from Tzaneen in September, whereas at Mohlaba the minimum is reached in June and breeding really never stops. At first sight, this extraordinary change of habit in two places only 12 miles distant appears to be incomprehensible. It has been found, however, that the climatic conditions of the two places are very different. This aspect of the problem will be elaborated in a later publication.

ADULTS. It is very commonly thought that ' species sanitation ' simply implies the correct identification of larvae, the identification and biology of the adult being neglected. This would be, of course, a perfectly correct procedure if biological behaviour could be measured by a morphological yardstick. It implies the principle that morphologically identical species—bearing in mind that there may be obscure structural differences of which we are unaware—always behave in the same way. That this is not so is, of course, well known in other countries. It is, therefore, possible that, by merely identifying larvae and proceeding with control in the shape of spraying or drainage, a harmless race of anopheline may be destroyed. This is especially possible if the amount and origin of malaria in a particular community is not first estimated—a proceeding which is not the rule in South Africa. To sum up, therefore, morphologically identical species may be divided into differing biological groups called 'races,' and, following the argument to its logical conclusion, 'race sanitation' may be substituted for 'species sanitation.'

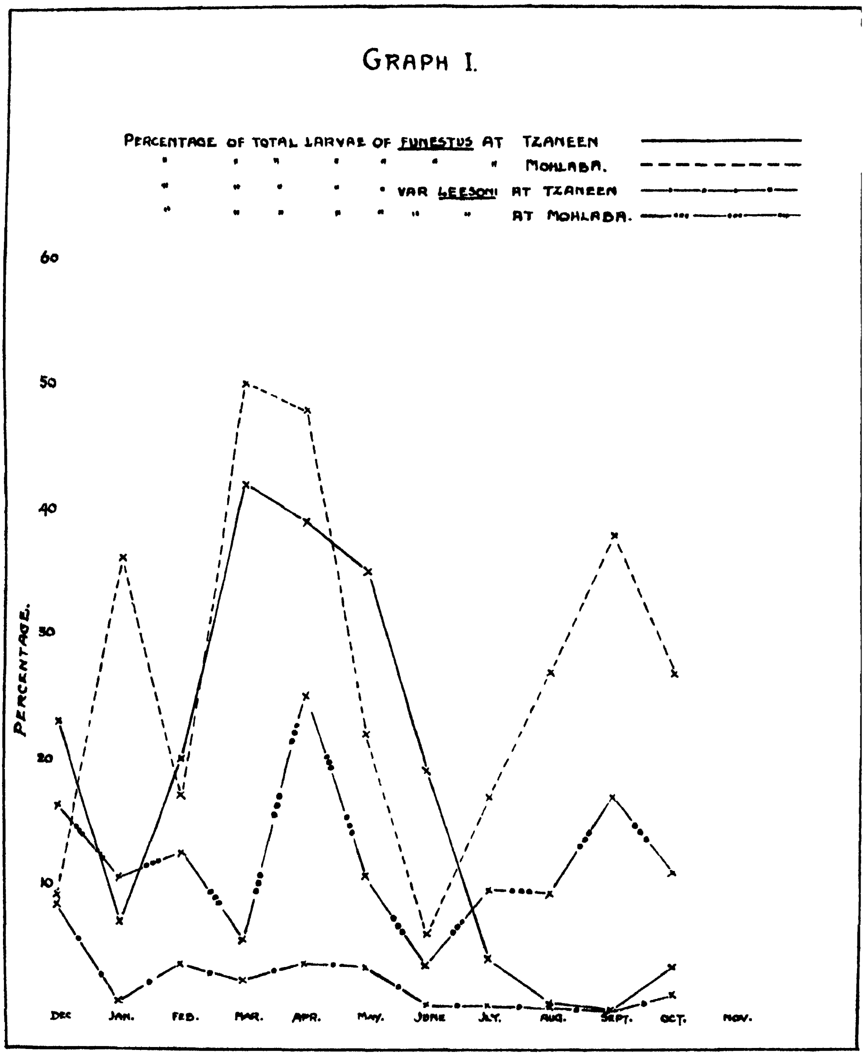


TABLE I. Tzaneen and Environs.

Month	*Funestus*	*Costalis*	*Funestus* var.	*Mauritianus*	*Maculipalpis*	*Rufipes*	*Pretoriensis*	*Longipalpis*	*Transvaalensis*	*Marshalli*	*Squamosus* var.	*Nili*	*Theileri*	*Natalensis*	*Squamosus*	*Rhodesiensis*	*Cinctus*	*Ardensis*	Total	% *Funestus* var. of total	% *Funestus* of total
Dec.	154	1	57	183	131	20	41	9	1	9	32	10	20	1	...	...	...	...	669	8·5	23
Jan.	10	...	1	13	89	...	6	...	16	9	...	...	...	...	...	...	...	...	144	·7	7
Feb.	72	...	12	36	152	14	5	1	4	4	34	2	19	...	...	...	...	...	355	3·6	20
Mar.	95	2	5	26	49	9	4	...	...	15	13	2	3	...	...	...	...	...	223	2·2	42
April	329	3	31	152	74	22	7	15	21	14	2	3	150	26	3	...	...	...	852	3·6	39
May	410	9	38	214	95	63	3	5	28	59	40	...	185	33	4	...	...	...	1176	3·2	35
June	195	...	1	345	55	41	...	2	32	96	74	...	100	22	32	1	3	8	1016	·09	19
July	32	...	1	266	31	50	...	1	21	83	158	...	8	136	7	3	...	...	797	·12	4
Aug.	4	...	1	200	11	3	...	...	47	99	193	...	31	99	8	...	...	...	696	·14	·6
Sept.	...	...	...	175	95	20	35	...	12	18	219	...	4	16	67	...	13	...	674	...	...
Oct.	17	...	6	85	98	38	94	1	16	14	64	...	10	2	23	...	...	...	468	1·3	3·6
Mohlaba and Environs.																					
Dec.	69	59	119	164	40	25	134	45	7	...	1	48	18	13	1	...	...	...	743	16	9
Jan.	38	...	11	19	7	2	23	3	...	...	...	5	6	1	...	...	1	...	104	10·5	36
Feb.	54	96	39	27	2	41	27	2	...	1	...	11	8	...	2	...	...	...	310	12·2	17
Mar.	55	2	6	7	...	14	...	...	5	9	...	5	3	...	2	...	...	2	110	5·4	50
April	195	9	101	16	...	6	4	1	1	3	...	9	24	35	...	...	...	1	405	25	48
May	113	79	54	96	3	2	26	21	2	1	4	...	90	13	11	...	...	...	515	10·48	22
June	46	319	28	87	...	5	18	150	3	40	...	...	61	18	34	...	...	1	810	3·45	6
July	114	6	65	100	...	2	42	46	25	41	...	...	74	57	109	...	3	...	684	9·5	17
Aug.	180	...	63	68	1	36	1	35	3	43	...	1	136	11	97	...	1	...	676	9·3	27
Sept.	228	...	104	120	...	11	16	...	...	...	...	...	75	9	40	...	...	...	603	17	38
Oct.	136	51	54	23	...	72	96	20	2	...	3	...	48	...	16	...	...	...	501	11	27

Recent researches in South Africa have clearly shown that differing biological behaviour within the same species is certainly to be reckoned with. It has already been pointed out that the prevalence of the larvae of *funestus* differs enormously in two localities. In one they are to be found all the year round, whereas in another they disappear during the winter. This is thought to be due to differing climatic conditions, so that the races, to distinguish them from purely physiological ones inhabiting similar climatic areas, may be called 'climato-races.' Now it has been found that at Mohlaba *funestus* adults enter human habitations much more readily than at Tzaneen. The actual figures show that 150 times more adults were caught per hut at Mohlaba than at Tzaneen over a period of 9 months. At the same time it must be remembered that at Mohlaba, owing to the large numbers present, only some adults were taken at each catch, whereas at Tzaneen a thorough examination was made of each hut, so that the figure is actually much bigger. It is therefore suggested that it will be found that the race of *funestus* which inhabits the Tzaneen area is largely zoophilic, whereas that of Mohlaba is largely homophilic.

Following from this it will at once be seen that it is essential, when contemplating anti-malaria control in a given area, to determine first of all the species present in human habitations. This brings us down to the identification of adult *funestus*. (It is assumed here that the vector of malaria is always a house-frequenter, and our experience so far has shown that the infectivity among non-house frequenting species is negligible in the parts of South Africa where detailed investigations have been made.)

The identification of adult *funestus* can be a very difficult and sometimes an impossible task. A scheme for separating it from other closely related forms has already been given by the author (1931), where it will be seen that the chief difficulty lies in the differentiation of *transvaalensis* and *funestus*. Further distinguishing features not given in the keys will be seen in the illustrations of the wings on pages 345 and 347 of the same publication. In *transvaalensis* the 4th, 5th and 6th veins are relatively paler, and this feature has been found to be of great use in separating some small specimens of *transvaalensis*. Further, in the latter species the base of the costa is broken by two pale spots, whereas in *funestus* there is at most one pale spot, and often this is absent. When we come to distinguish *funestus* var. *leesoni* from *funestus,* however, a very serious difficulty arises, for it has been found that the females are inseparable. Close on 50 specimens of the variety have been bred in our laboratory from identified larvae, and in none of these has any feature been found to distinguish them from *funestus*. As a general rule, the pale markings on the wing of the variety are more restricted, but, as is well known from the work of Leeson (1930), the wing of *funestus* is very variable. That author mentions 9 types of wing variations, and we give here 6 others (fig. 7) not mentioned by him. It is of

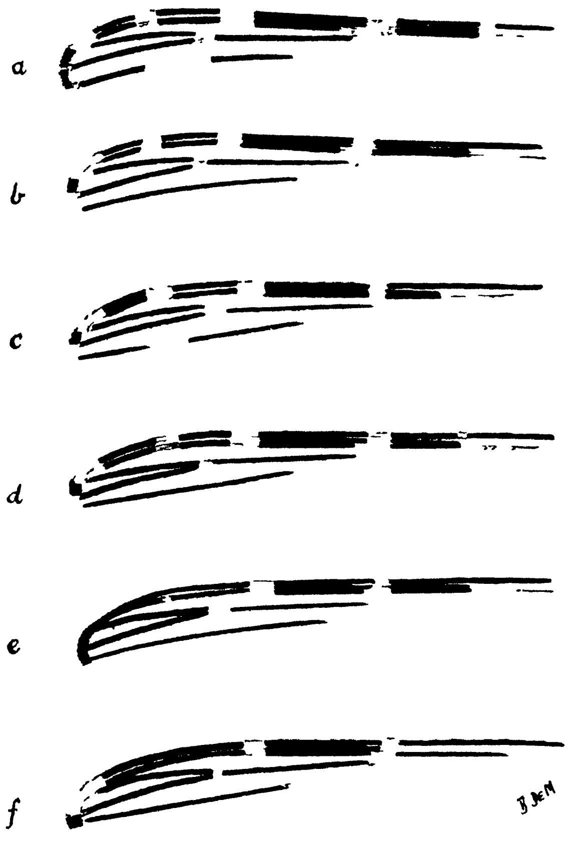


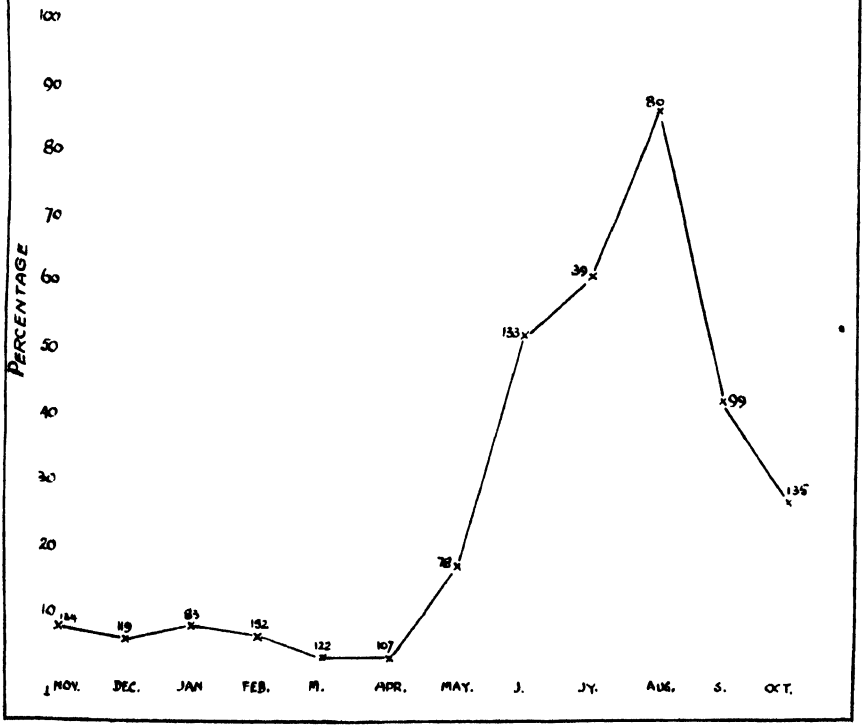
FIG. 7. *A. funestus*, costa, subcosta, first, second and third veins of the wing. *a.*—base of the costa broken, third vein pale in centre, *b*—base of costa unbroken, third vein dark, *c.*—base of costa unbroken, third vein pale in centre, *d* base of costa broken, third vein dark; *e.*—base of costa unbroken, third vein all dark, apex of wing all dark, other pale spots reduced; *f.*—base of costa unbroken, third vein all dark, apex of wing largely pale.

interest to note that, whereas the form with the base of the costa broken apparently does not appear in Southern Rhodesia, it is quite common down here. In none of our specimens of *funestus* var. *leesoni*, however, is the base of the costa broken. Leeson has further

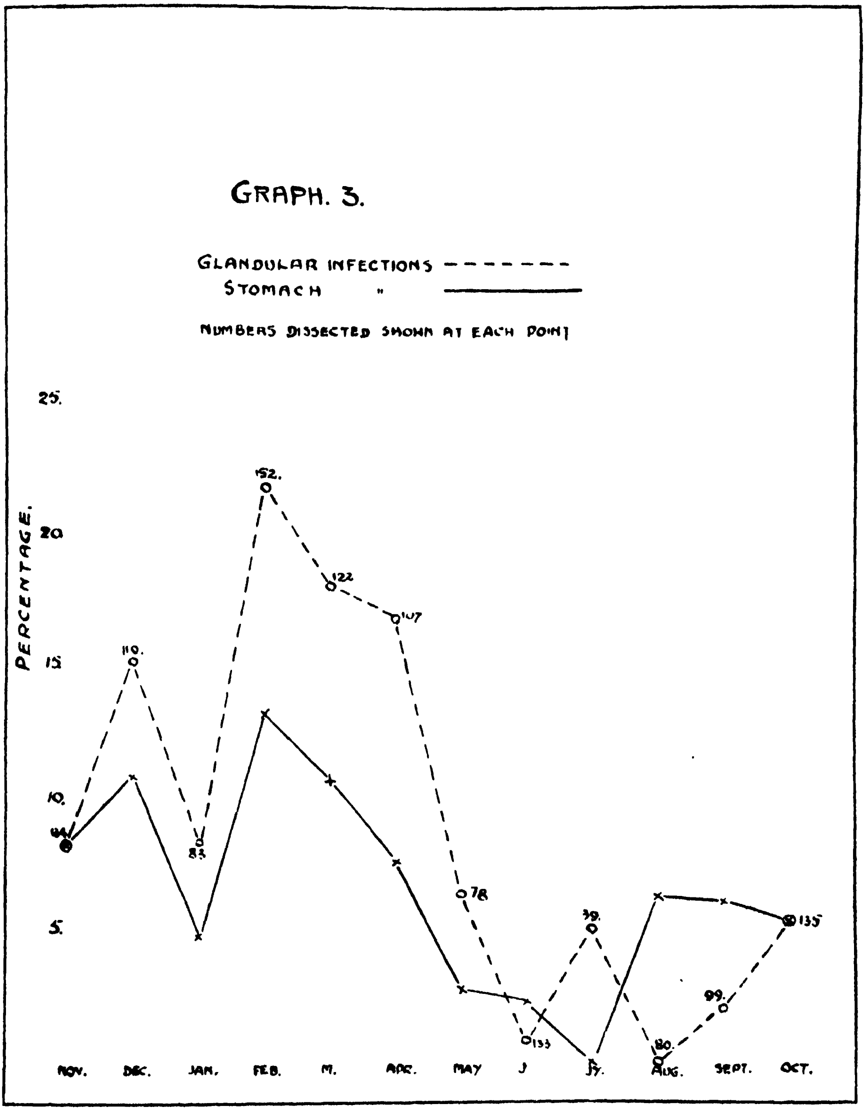
GRAPH. 2.

PERCENTAGE FUNESTUS DISSECTED WITH THIRD VEIN ALL DARK

NUMBERS DISSECTED SHOWN AT EACH POINT.



noted that the type of *funestus* with the third vein pale is the wet season form, and this we have been able to confirm. Graph II shows the percentage of specimens dissected during a year, with the third vein totally dark. We have, however, been unable to find any evidence that this form is the hibernating one, as was suggested by Leeson. It is suggestive, nevertheless, that in the month of August, when the percentage of dark winged forms reached its maximum, there were no glandular infections noted among 80 specimens. Graph III gives the infection rate among *funestus* adults caught in selected huts at Mohlaba.



A. funestus var. *leesoni* cannot definitely be said to show the same seasonal variation, although out of 50 females, 5 had the third vein all dark, and 4 of these occurred in May, a relatively dry month.

The males of *funestus* var. *leesoni* are easily distinguished from *funestus* by having 3 instead of 2 pale markings on the palp (fig. 8, *a* and *b*).

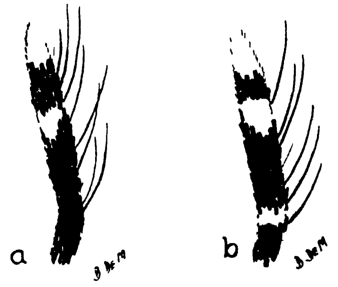


FIG. 8. *a.—A. funestus*, apex of male palp; *b.—A. funestus* var. *leesoni*, apex of male palp.

It will be clearly understood from the above that it is impossible to estimate directly the rôle played by *funestus* var. *leesoni* as a vector of malaria. We have, however, abundant indirect evidence that this variety does not enter human habitations to any extent. During the course of the year, 237 males of *funestus* have been taken in our catching stations, and only 1 male definitely belonging to the variety. Other evidence which shows that this variety is not homophilic is suggested by the following experiment. During April, 1932, *funestus* and its variety were breeding in enormous numbers in the Letaba River at Tzaneen. A tent was pitched on the bank of the river, and a native slept in it as bait on 7 nights. Each morning a catch of adults in the tent was made, as well as during the night or evening, when mosquitoes were noticed. The total catch was *funestus*, or *funestus* var. *leesoni*, 2 females; *funestus* var. *leesoni*, 1 male; *mauritianus*, 2 females; *theileri*, 3 females; *costalis* (*gambiae*),* 2 females. Compare this with a catch on the Letsitele River at Mohlaba, where, during a single night, 32 females of *funestus* typicus or var. and 12 males of *funestus* typicus entered the same tent, showing that the tent and its occupant were not distasteful to a homophilic strain.

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* The name *costalis* is used in this Journal.—EDD.

GEOGRAPHICAL DISTRIBUTION OF *A. FUNESTUS* var. *LEESONI*

We have records of this species from the following places:—Nylstroom, Waterberg District, Transvaal; Louis Trichardt, Zoutbansberg District, Transvaal; Letaba District, Transvaal; N'Kwaleni Valley, Natal; Darnall, Natal.

I am greatly indebted to Dr. Annecke and his staff of the Department of Public Health, and to my assistant, Mr. Meeser, for the great help rendered in accumulating the data for this paper.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

I. A REVIEW OF EXISTING KNOWLEDGE ON THIS SUBJECT, WITH SOME GENERAL OBSERVATIONS

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CONTENTS.

		PAGE
I.	Introduction	99
II.	On the Dissection of the Salivary Glands in Tsetse	104
III.	On Double Infections	107
IV.	Field and Laboratory Records of the Transmitting Power of Tsetse	109
V.	General Inferences	117

I. INTRODUCTION

Prominent among the problems that still intrigue the student of the African trypanosomes is the imperfection of their adaptation to the tsetse upon which they depend for survival in nature. It has long been recognised that the so-called pathogenic trypanosomes fall readily into three groups according to their disposition in the fly. One group develops exclusively in the proboscis, another invades both gut and proboscis, and the third, in which are the two trypanosomes of man, utilises the gut and salivary glands. The three groups differ in their ability to develop cyclically in *Glossina*. In a sample of, say, 500 or 1,000 wild flies of any of the common species of game tsetses, the *vivax* or proboscis-only group will be found to be the most common, followed in order of frequency by the *congolense* or proboscis-and-gut group, and, at a considerable distance, by the *brucei* group, comprising *T. rhodesiense* and *T. gambiense.*

In an adequate sample of wild flies it is rare to find more than 1 or 2 per thousand carrying the infective forms of the *brucei* group, although 20 or 30 per cent. of the game from which the flies derive their main food supply may be proved to be carrying these trypanosomes.

The same strange inability to develop in the great majority of tsetse flies to which they are exposed characterises the behaviour of these trypanosomes in experiments with flies hatched in the laboratory. The percentage of infected flies is, of course, higher than among wild flies, because every fly takes up quantities of trypanosomes during the period of the infecting feeds. But even here, in the majority, often the very great majority, of the flies no development takes place.

The *vivax* group in wild flies has been shown sometimes to attain a 40 or even 50 per cent. infection rate (Lloyd *et al.*, 1924). Experimental data on the transmission of this group with laboratory-bred flies are very meagre, but no doubt in the more favourable conditions attainable in the laboratory still higher figures would be obtained. In their restricted adjustment to the insect intermediary, the *brucei* group of trypanosomes presents a remarkable contrast to certain non-pathogenic organisms such as *T. melophagium* and *T. lewisi*, parasites of the sheep and rat respectively, which in the appropriate insect host, the ked and the flea, produce on occasion 100 per cent. infections.

The difficulty of infecting tsetse with the *brucei* group of trypanosomes has attracted attention ever since the early days of research into trypanosomiasis, when, in 1909, Kleine, and immediately afterwards Bruce and his colleagues, established the existence of a cycle of development in the tsetse.

To account for the phenomenon, a number of explanations have been advanced. Bagshawe and also Kleine and his colleagues have laid stress on the probable influence of climate in explaining discrepancies between the results of transmission experiments with the same trypanosomes and the same species of tsetse in different parts of central and eastern Africa. Roubaud (1920) invoked racial differences in the tsetse, some races being better transmitters than others. Kleine and Eckard developed this theme. They compared the transmitting power of tsetse whose parents had been maintained on a diet of blood containing numerous trypanosomes, with that of the offspring of flies fed on clean blood. No difference was discernible. Kinghorn, Yorke and Lloyd (1912) reached the conclusion that, whereas the first part of the developmental cycle of *T. rhodesiense* in *G. morsitans* could take place at comparatively low temperatures (60° F.), the final invasion of the salivary glands demanded higher temperatures (75° to 85° F.). Variations in relative humidity they found to be unimportant, even such contrasts as 70 to 72 per cent. and 36 per cent. exerting no appreciable effect on the developmental cycle.

In 1912, Muriel Robertson propounded the view that *T. gambiense* undergoes a recurring endogenous cycle of development in the blood of the mammal, in the course of which 'there are definite periods when the blood is not infective to fly although trypanosomes are present.' Her studies led her to conclude that 'the short forms of the trypanosome in the mammal are those destined to carry on the cycle in the transmitting host' (Robertson, 1913). It was my privilege to watch the progress of this interesting work at Mpumu Laboratory in Uganda, and for a number of years now evidence has been slowly accumulating on the adequacy of this view to explain daily variations in the number of flies that develop flagellate infections in experimental transmissions with trypanosomes of the *T. brucei* group. The subject is beset with difficulties. Experimental transmissions require much time, an almost unlimited supply of tsetse and a considerable share of good fortune; and a system of control experiments, so simple of application in many of the problems of bacteriology, is here often almost impracticable. Data bearing on Robertson's views will be considered in a later paper of this series.

It seems certain that in nature strains of trypanosomes undergo frequent modifications as they pass from host to host. Among the game of a fly belt will be found individuals possessed of degrees of tolerance or immunity acquired by repeated exposure to infection; and, although we have still much to learn about the immunology of trypanosomiasis, there is already some justification for supposing that passage through such an animal may leave an impress on the strain as a whole. Then, too, admixture in the same animal of different strains of the same species, or of one or more different species of trypanosome must be a common occurrence in nature, and this again will help to keep the kaleidoscope in motion.

After a brief excursion into this line of work, Lloyd reached the conclusion that ' the factors which influence the rate of infection in tsetse by trypanosomes are so many that it is impossible to estimate with any accuracy the transmissibility of any particular strain ' (Lloyd, 1930).

I do not quite agree with this verdict. Great accuracy is not at present possible, nor is it necessary to our purpose. But it is certainly possible to distinguish between a readily transmissible and a non-transmissible strain, and also to recognise broad differences between different transmissible strains. Once they are safely isolated at the laboratory, strains of the *brucei* group will, I think, be found on careful study to exhibit fairly definite characters in their behaviour in the tsetse intermediary. Some are more, some are less readily transmissible, and in constant conditions of maintenance behave in a manner consistent with this description. A strain may show different degrees of transmissibility in different species of mammalian hosts, in the manner indicated in the Final Report of the League of Nations Commission ; but here again the variation appears to follow definite rules.

There is also a tendency, in certain conditions of maintenance, for a strain to lose its transmissibility by *Glossina*. Hitherto this phenomenon has been observed chiefly in *T. gambiense*, and the transmissibility of this species by tsetse seems to be a distinctly unstable character. Progressive loss of biological contact between trypanosome and tsetse is characterised by a stage where invasion of the salivary glands no longer occurs, although the trypanosome can still develop readily in the gut ; and, according to a steadily growing experience with *G. palpalis* in Uganda, in both field and laboratory, once the power of invading the glands has been lost it is never recovered.

Indications of this kind, vague though they may be at first, gradually take shape and encourage and suggest further investigation.

Our situation at Entebbe is extremely favourable to the study of the transmissibility of trypanosomes by *G. palpalis*, whose pupae are easily obtained in large numbers. It is hoped also in future to secure from time to time adequate supplies of *G. morsitans* pupae, a number of which have been sent to us recently by Dr. C. H. N. Jackson, of the Tsetse Reclamation Department, Tanganyika

Territory. Our aim is to obtain as many strains as possible of polymorphic trypanosomes and to test them in clean tsetse as soon as possible after their isolation from either wild fly or game or man. The method of testing is uniform, and it is claimed that the results obtained are comparable. After a brief study of their behaviour in the fly, the majority of the strains are for reasons of economy abandoned as new ones are acquired. In a few instances, the behaviour of a strain is followed for some months, during a series of cyclical passages through fly from monkey to monkey. These laboratory studies, supported by field investigations in accessible fly belts in and around Uganda, render possible the gradual accumulation of information that must eventually lead to an increased understanding of the polymorphic group of trypanosomes and their susceptibility to measures of control.

It is proposed, in the series of papers of which this is the first, to consider in turn the more obvious of the factors that go to make up the environment of the *brucei* group of trypanosomes in nature. The object is to discover whether anything really does exert an influence on their infectivity to tsetse, or, otherwise expressed, on the power of the fly to transmit them. If nothing can be found, it may nevertheless be possible, by a sufficiently exhaustive examination, to eliminate some of the uncertainty that at present obscures this subject.

In the present paper, a general review will be taken of the results obtained by different observers by the dissection of game tsetse in various parts of Africa.

By this means it is hoped to secure a conception of the degree of adaptation between trypanosome and fly attainable under natural and under laboratory conditions. The available literature has been searched and every observation bearing on the matter extracted and recorded. In several instances, the cited percentage of infected flies differs from that of the original author, the discrepancy being due to the inclusion in the original estimate of a number of flies that were never actually dissected.

In many of the transmission experiments with game tsetse, no details of dissections are recorded, and in others only a few of the flies used were examined.

It is not necessary for our purpose to attempt to distinguish between flies with infected glands and flies which merely have flagellates in the intestine and are consequently not infective. The important figure is the percentage of flies that contain established and developing flagellates. The main object of this paper is to collect and present the data on this subject, accumulated by different observers.

There is a tendency nowadays to condemn and discourage what is called academic or long range research, on the grounds that the British tax-payer demands only results of immediate practical utility. The Entebbe Institute has in the last year or so come in for its full share of this kind of criticism.

It may, therefore, be expedient to point out that the kind of research exemplified by the investigations to be recorded in this series of papers does not represent the sole or even principal part of the programme we are attempting to carry out. Much of the evidence to be presented in this series has been supplied by transmission experiments directed to the solution of other problems of unquestionable practical importance. As far as possible, all passages from animal to animal of the trypanosomes studied at the Institute are made by cyclical transmission by tsetse. Large numbers of flies are employed in these undertakings, and, by slightly modifying the ordinary procedure, it is easy to attend to a number of side issues *en route* to the main object.

On the other hand, it may reasonably be contended that any addition to the sum of knowledge about the normal relations of the human trypanosomes to their tsetse vectors is worth both time and trouble. Even a 'negative' result, if properly controlled and substantiated, may have real practical value in dealing with the spread and control of human trypanosomiasis.

II. ON THE DISSECTION OF THE SALIVARY GLANDS OF TSETSE: UNILATERAL INFECTIONS

Kinghorn, Yorke and Lloyd, in the Eighth Interim Report of the Luangwa Valley Commission, after describing their method of dissection of the salivary glands, remark: 'This method has obvious advantages over that described by the Royal Society Commission, in which after snipping off the terminal segment of the abdomen, the whole contents were expressed on to a glass slide and the salivary glands subsequently separated from the mass of intestines and other structures. We claim for the technique adopted by us that the process is quicker, more certain, and that the danger of contamination from the intestines is reduced to a minimum.' This calls for a word of explanation. Whatever was the method first employed by the Royal Society's Commission, that in use in Uganda during recent years does not aim at extracting the glands simultaneously with the rest of the abdominal contents through the posterior opening. By a very simple manipulation of the needles, the abdominal contents, sometimes accompanied by the proventriculus, are expressed first, and then, if they are wanted, the glands are pulled out separately. With very little practice, it is easy to obtain both glands and their ducts intact as far as the extreme proximal end of the latter. This is important, not from any danger of confusing gut forms with salivary gland flagellates, but because, once a gland is ruptured anywhere along the main tubular part, its contents are liable to extremely rapid evacuation through the opening. Light infections of a gland with trypanosomes—and such infections are much commoner than is generally realised—may be completely emptied through such a wound in a surprisingly short time, and the scanty flagellates that emerge are easily lost to view.

A heavy gland infection is at once obvious, and on their emergence through a rupture or through the broken extremity of the duct the masses of gland flagellates are easily recognisable, wherever they may wander. But in the case of light infections very careful inspection is necessary all along the organ, which must be under sufficient pressure from the coverslip to ensure perfect optical section. In such cases it is advisable first of all to inspect the orifice of the duct, from which it may be possible to watch flagellates emerging under pressure. Then, by increasing the pressure on the coverslip, a rupture can be made, and again the contents carefully scrutinised as they flow out. The method of deliberately cutting off the fly's abdomen and so severing the glands at the junction of the glandular part with the broader part of the ducts is, I think, open to the grave drawback that certain types of light infection might easily be lost by evacuation through the incision. I have seen gland infections with only a few flagellates in the duct and none in the glandular portion; others, where the whole gland and duct is blocked with them ; and others, again, where there are a few scattered flagellates in the distal part of the duct and the shoulder, none in the middle portions of the glands, and a few attached in the extreme fundus. It will be shown in a later paper that there is good reason to conclude that light gland infections are characteristic of certain strains.

And here may be described what is, I believe, a hitherto unrecorded phenomenon, namely unilateral gland infection, where only one of the glands contains flagellates.

I have now witnessed this phenomenon on three occasions in laboratory-bred flies infected experimentally at Entebbe. In a fourth fly, marked asymmetry occurred.

(1) Experiment 99. A ♀ G. *palpalis :* killed on 31st day of experiment : *T. rhodesiense* strain. One gland absolutely negative; the other contained a moderate infection of duct, body and fundus. The negative gland was emptied and the contents carefully scrutinised. Dissection perfect.

(2) Experiment 916. A ♀ G. *morsitans :* killed on 38th day of experiment : *T. rhodesiense* strain. One gland negative from tip of duct to fundus; contents expelled and scrutinised. Dissection perfect. The other gland showed a sparse and straggling line of flagellates attached to the duct ; a narrow, thickly infected zone, free forms and attached, at the shoulder and in the anterior half of the body ; and none in the posterior half of the gland.

(3) Experiment 970. A ♂ G. *morsitans :* killed on 43rd day of experiment : *T. rhodesiense* strain. One gland heavily infected throughout its whole length. The other gland, intact from the tip of duct to fundus, showed a few flagellates attached in the narrow distal part of the duct only ; none in the glandular part or fundus. Dissection perfect ; contents expressed and examined.

(4) Experiment 974. A ♂ G. *morsitans :* died on 28th day of experiment : *T. rhodesiense* strain. One gland showed a moderate infection of duct and shoulder and body. The other gland, perfectly dissected, was negative from end to end.

Of these 4 flies, numbers 1 and 4 were not tested on clean animals, number 2 infected several clean animals, and number 3 the only one it bit. The 3 G. *morsitans* form part of a total of 26 flies of this species which developed gland infections in various transmission experiments at Entebbe.

Before excluding a gland infection, it is thus necessary to see both glands throughout their length.

During the last few months, thanks to the kindness of Mr. Swynnerton and Dr. Jackson, of the Tsetse Research Department, Tanganyika, I have had the opportunity of carrying out transmission experiments with G. *morsitans* hatched in the laboratory. Compared with G. *palpalis*, this tsetse is generally speaking of a more delicate build. The breaking strain of the glands is, for example, considerably less in G. *morsitans* than in *G. palpalis*, and it is in consequence more difficult to pull the glands out intact through the posterior amputation wound.

For this reason, the dissection of the glands of all but freshly killed *G. morsitans* is more difficult, and a modified procedure has been adopted. If, after removal of the gut, fat body, etc., the ends of the glands do not protrude posteriorly through the abdominal incision, the head of the fly should be gently pushed off the thorax, in the manner described by Lloyd, and the glands drawn out anteriorly. I find it better to pull the thorax off the glands by anchoring the head, than to pull the head and glands away from the anchored carcase. This modification is very useful in dissecting flies that have died over or during the night. With a freshly killed *morsitans* the glands usually come out readily by the ordinary Uganda method. If they do not, then the head method can be employed.

III. ON DOUBLE INFECTIONS

The first reference to the possible occurrence of double infections with two species of trypanosome in the same individual fly occurs in the First Report of Johnson and his colleagues at Sherifuri (Johnson and Lloyd, 1923). No details are given, but the phenomenon is mentioned as complicating the calculation of the trypanosome infection rate in wild flies as determined by dissection. In their Second Report, double infections are referred to at greater length, and a very interesting observation is recorded (Lloyd *et al.*, 1924). In all, 7 gland infections were detected in 19,000 wild *morsitans* and *tachinoides* dissected. In 6 out of these 7 flies, fully developed infections of the *congolense* group were also recognised (Lloyd *et al.*, 1924). The same phenomenon was met with by me during the dissection of wild *G. swynnertoni* in the neighbourhood of Mwanza in Tanganyika Territory, where a fly was found with a massive infection of gut, glands and proboscis. In the estimate of infected flies for the area, this fly was counted as infected with *brucei* group (Duke, 1923).

There will be occasion to refer again to these 8 flies in a later paper of this series. It may, however, be noted in passing that they afford very significant evidence that certain individuals among the tsetse population are specially fitted to act as intermediate hosts of the pathogenic trypanosomes, and that the peculiar endowment, whatever it may be, appeals to at least two, and probably to all three, groups of these trypanosomes.

In the present discussion, double infections are mainly of interest because of the confusion they may introduce into the estimation of the infectivity of wild fly.

Take, for example, the 7 doubly infected flies mentioned above. In a calculation of the percentage of wild flies that carry trypanosomes—the point with which we are concerned in this paper—they count merely as 7 infected flies. But in a calculation of the prevalence of trypanosomes in wild fly, they present 14 infections.

In the reports of the West Coast investigators, it is not sufficiently clear whether the tables of the results of dissection of wild flies given in the first report and in Lloyd and Johnson's later paper relate to the same or to different collections of flies. The Table in the First Report apparently does not differentiate between simple and double infections : presumably a doubly infected fly would be recorded as one infected insect. In the other Table, it is impossible to determine the percentage of flies carrying trypanosomes, owing to inclusion of double infections under each of the groups of trypanosomes represented. For our purpose, therefore, the percentage given in the First Report will be taken. The West Coast figures are considerably higher than those obtained by other observers. This is in part due, no doubt, to the more elaborate technique rendered possible by bringing the flies to the laboratory for dissection. My own dissections were all done single handed, save for the removal, by a native, of the legs and wings of the flies, whilst I was on safari in the fly-belts. The method pursued was to remove the gut and the proboscis of each fly, and to examine them in saline under coverslips, one at each end of the slide. If the gut and proboscis are both positive, the fly is recorded as showing a *congolense* infection ; if flagellates are found in the gut alone, then, and then only, are the glands examined. With this technique it is plain that double infections would normally be overlooked, and my figures of the incidence of the *brucei* group will tend to err on the low side. The single instance in *G. swynnertoni*, referred to above, was detected simply because the fly had bitten me and my attendant, and curiosity was therefore aroused.

IV. FIELD AND LABORATORY RECORDS OF THE TRANSMITTING POWER OF TSETSE

In this Section, the observations on the infectivity of tsetse recorded in the available literature are recorded in two Tables, I and II. Some figures are included from the Entebbe records, for the sake of comparison. Table I relates to wild fly, Table II to experiments in the laboratory with the clean tsetse hatched in captivity. In Table I, the total number of flies infected with all three groups of pathogenic trypanosomes is given. In Table II, the figures relate solely to the polymorphic group.

Although it contains practically all recorded observations on this subject, there is little to be learnt from Table II, partly because of lack of information about the strains of trypanosomes tested, and partly because the great majority of the experiments deal with very small samples of flies.

In general, the figures agree with those shown in Table I, and from the two Tables it would appear that a 20 per cent. infection rate in any of the species of tsetse included in the inquiry is an unusual occurrence.

In Lloyd's experiments with laboratory-bred *tachinoides*, there occurs a single experiment (No. 44) comprising 15 flies, 13 of which, or 86·6 per cent., were infected with *T. brucei* (Lloyd, 1930). This, as Lloyd remarks, is the highest infection rate hitherto recorded. These particular flies had been kept in the incubator at 92° to 97° F. for 8 days. An attempt to repeat the experiment under identical external conditions produced only 8 per cent. of infections. In the 277 flies used in the whole test, in which No. 44 was included, 9·3 per cent. were infected. Experiment No. 44 must, therefore, be regarded for statistical purposes as a freak sample of flies, and stands unexplained, a most interesting and noteworthy observation. That in certain as yet undetermined circumstances unusually high infection rates may occur is shewn also by Corson's observation that, of 14 wild *G. pallidipes* which had been fed for a few weeks on a sheep infected with *T. rhodesiense*, 5 (i.e. 35 per cent.) were found on subsequent dissection to carry gland infections, another arresting observation in this line of work.

Both these records suggest that at times tsetse may, even with the polymorphic group of trypanosomes, approach the ideal of the

TABLE I

Observer	Date	Altitude (feet above sea level)	Place	Species of fly	Number dissected	Per cent. infected	Remarks
Kinghorn and Montgomery (1909)	1909	...	Kambole, south end of Lake Tanganyika	*morsitans*	113	7·8%	...
Taute (1913) ...	1912–13	1,800 ft.	Lubimbimu, Tanganyika Territory	*morsitans*	369	7·3%	...
Bruce, *et al.* (1915)	1912	3,000 ft.	Upper Shiré, Nyasaland	*morsitans*	1,975	6·53%	...
Bruce, *et al.* (1915)	1913	3,000 ft.	Upper Shiré, Nyasaland	*morsitans*	1,060	8·58%	...
Bruce, *et al.* (1915)	...	3,000 ft.	Upper Shiré, Nyasaland	*brevipalpis*	496	8·8%	(1·6% *T. grayi*).
Robertson	1914	3,300 ft.	Masindi, Uganda	*morsitans*	445	9·4%	Proboscis only ... ... 3·8% Proboscis and gut ... 2·9% Gut only ... ... 2·7%
Duke (1916) ...	1914	3,300 ft.	Masindi, Uganda	*morsitans*	1,117	13·4%	Proboscis only ... ... 8·5% Proboscis and gut ... 3·1% Gut, not proboscis ... 1·8% (3 flies with salivary glands). No *T. grayi*.
Duke (1916) ...	1914	2,030 ft.	Fajao, Uganda	*morsitans*	606	9·6%	Proboscis only ... ... 7·0% Gut and proboscis ... 1·8% Gut only ... ... 0·8% No *T. grayi*.
Duke	1932	2,030 ft.	Fajao, Uganda	*morsitans*	735	11·9%	Proboscis ... ... 3·5% Proboscis and gut ... 1·4% Gut only (mammal.) ... 3·0% *T. grayi*... ... ... 3·8%
Duke (1916) ...	1914	2,800 ft.	Ngussi River, Uganda	*pallidipes*	65	18·4%	Proboscis ... ... 1·5% Gut only ... ... 7·6% Proboscis and gut ... 6·3% Gut and salivary glands 3·0% No *T grayi*.
Duke	1932	2,800 ft.	Ngussi River Uganda	*brevipalpis*	121	0·8%	...
Duke (1923) ...	1923	3,700 ft.	Near Mwanza, Tanganyika Territory	*swynnertoni*	2,206	9·3%	Probocis only ... ... 6· Proboscis and gut ... 1· Gut only ... ... 0· Gut and gland ...0·1 No *T. grayi*.
Duke	1932	4,000 ft.	Nsongezi, Uganda	*morsitans*	109	5·5%	Proboscis ... ... 0· Proboscis and gut ... 1· Gut ... ... 2·7%
Duke and Wallace ...	1932	3,700 ft.	Bugoto	*pallidipes*	231	6·4%	Including *T. grayi* ... 1·7%
Johnson and Lloyd (1923)	1922-23	...	N. Nigeria	*morsitans*	500	26·3%	Proboscis ... ...15·2% Proboscis and gut ... 6·0% Gut only ... ... 5·2% No *T. grayi*.
Johnson and Lloyd (1923)	1922-23	...	N. Nigeria	*tachinoides*	1,500	11·3%*	Proboscis ... ... 5·4% Proboscis and gut ... 1·1% Gut only ... ... 4·8%

* In a sample of 1,497 *G. tachinoides*, 11·9% were found infected with *T. grayi*. The same sample contained 11·9% of flies carrying developmental forms of mammalian trypanosomes, i.e., the total percentage of *G. tachinoides* infected with trypanosomes, mammalian and reptilian = 23·8%.

successful insect vector, i.e. 100 per cent. of successes. Unfortunately, in both instances the figures are far too small to justify any conclusion, save perhaps that extreme caution is required in carrying out experiments on this subject. Records of 25 per cent. and even 30 per cent. infected do indeed occur in single transmission experiments with *G. palpalis* and *T. rhodesiense* at Entebbe. But here also these high percentages always relate to very small samples, and the figures for the whole test invariably assume more sober proportions.

My own experience, much of which will appear in later papers of this series, strongly indicates that *G. morsitans* is a better transmitter of trypanosomes than *G. palpalis*. But, so far as the experimental investigation of this point has yet progressed, the difference is not very great; and a 20 per cent. infection rate in tsetse exposed to the polymorphic group of trypanosomes still remains a standard rarely realised, either in nature or in the laboratory.

There is evidence also that *G. pallidipes* may in certain localities show a higher infection rate than *G. morsitans*. The figures for this species in the Ngussi area in 1914 (18·4 per cent.) are higher than any obtained with *G. morsitans* in Uganda; and 3 per cent. of the flies then dissected showed infection of the salivary glands, which is, I believe, the highest rate ever reported for the polymorphic group in wild tsetse. Corson's experiment quoted above is another instance of the capability of *G. pallidipes*. At Fajao in Uganda, in 1932, out of 5 *G. pallidipes* caught and dissected, 2 were infected, one in the proboscis and the other in the gut. On the other hand, the *pallidipes* dissections from the shores of Lake Victoria, shown in Table I, reveal a low infection rate, and it may be that climate plays an important part in determining the efficiency of this species as a transmitter of trypanosomes. It must be remembered, however, that on Lake Victoria *G. pallidipes* is closely associated with hippo, and the presence of *T. grayi* shows that the fly also feeds freely on reptiles.

I have no longer any doubt that strains of trypanosomes differ from one another in their transmissibility by tsetse, and my experience points to the conclusion that, as a general rule, *T. rhodesiense* is more readily transmitted by *Glossina* (even by

TABLE II

Author	Date	Altitude above sea level	Place	Species of trypanosome	Species of fly	Number dissected	Number +	Per cent.	Remarks.
Rodhain, *et al.* ... (1913)	1910	2,250 ft.	Belgian Congo Sinkisa	*gambiense*	*G. morsitans*	171	16	9·3	...
Kleine (1909) ...	1909	4,000 ft.	Tanganyika Kirugu	*gambiense*	Probably *G. swynnertoni*	120	2	1·6	...
Kleine (1911) ...	1911	2,550 ft.	Niansa, Tanganyika	*gambiense*	*G. morsitans*	282	...	7·8	...
Kleine (1912) ...	1911 and 1912	2,550 ft.	Niansa, Tanganyika	*gambiense*	*G. morsitans*	60	10	16·6	...
Kleine (1912) ...	1912	3,700 ft.	Schirati, Tanganyika	*gambiense*	Probably *G. swynnertoni*	400	...	2·5	...
Kleine (1913) ...	1913	3,000 ft.	Between Tabora and Udjiji, Tanganyika	*gambiense*	*G. morsitans*	881	54	6·1	...
Kinghorn, Yorke and Lloyd (1912)	1911–12	1,900 ft.	Nawalia, Rhodesia	*rhodesiense*	*G. morsitans*	39	5	12·7	...
Kinghorn, Yorke and Lloyd (1912)	...	1,900 ft.	Nawalia, Rhodesia	*rhodesiense*	*G. morsitans*	132	27	20·4	Of these + flies, 5 showed scanty infections and died on the 4th, 5th, 6th, 7th and 9th days of the cycle: excluding these as "passive survival infections" we get 16·6%.
Kinghorn, Yorke and Lloyd (1912)	...	1,900 ft.	Nawalia, Rhodesia	*rhodesiense*	*G. morsitans*	43	1	2·5	...
Bruce, *et al.* (1915)	1912–13	3,500 ft.	Kasu, Nyasaland	*brucei*	*G. morsitans*	308	47	15·3	...
Bruce, *et al.* (1915)	1912–13	3,500 ft.	Kasu, Nyasaland	*brucei*	*G. morsitans*	215	14	6·5	...
Bruce, *et al.* (1915)	1912-13	3,500 ft.	Kasu, Nyasaland	*rhodesiense*	*G. morsitans*	287	25	8·7	...

TABLE II—*Continued.*

Author	Date	Altitude above sea level	Place	Species of trypanosome	Species of fly	Number dissected	Number +	Per cent.	Remarks.
Duke	1932	4,000 ft	Entebbe	*brucei* (1)	*G. morsitans*	147	16	10·8	...
Duke	1932	4,000 ft.	Entebbe	*brucei*	*G. morsitans*	125	8	6·4	...
Duke	1932	4,000 ft.	Entebbe	*brucei* (2)	*G. morsitans*	167	11	6·5	
Duke	1932	4,000 ft	Entebbe	Several different	*G. morsitans*	2,238	157	7·7	This total represents [illegible] *G. morsitans* transmissions done [illegible] Entebbe up to date with *T. br*[illegible] and *rhodesiense* : 70 of the strains were non-transmissible.
Lloyd (1930) ...	1929	. .	Sherifuri, N. Nigeria	*brucei* (C	*tachinoides*	206	5	2·4	...
Lloyd (1930) ...	1929		Sherifuri, N. Nigeria	*brucei* (D	*tachinoides*	149	1	0·6	...
Lloyd (1930) ..	1929	.	Sherifuri N. Nigeria	*brucei* (C D)	*tachinoides*	129	3	2·3	...
Lloyd (1930) ...	1929		Sherifuri, N. Nigeria	*brucei* (C), (D), separate feeds	*tachinoides*	277	26	9·3	Includes experiment 44, in which 13 out of 15 flies were +, i.e., 86·6%. This experiment had been kept at 92-97 °F. for 8 days.
Duke	1932	4,000 ft	Entebbe	*rhodesiense* (1)	*palpalis*	28	26	9·0	...
Duke	1932	4,000 ft	Entebbe	*rhodesiense* (2)	*palpalis*	188	16	8·5	...
Duke	1932	4,000 ft	Entebbe	*rhodesiense* (3)	*palpalis*	627	47	7·6	...
Duke	1932	4,000 ft	Entebbe	*brucei* (1)	*palpalis*	760	43	5·6	...
Duke	1932	4,000 ft.	Entebbe	*rhodesiense* (4)	*palpalis*	238	43	18·0	...
Duke	1932	4,000 ft	Entebbe	*rhodesiense* (5	*palpalis*	648	59	9·1	...
Duke	1932	4,000 ft	Entebbe	*rhodesiense* (6)	*palpalis*	774	68	9·3	...
Duke	1932	4,000 ft.	Entebbe	*rhodesiense* (2)	*palpalis*	297	49	16·5	...
Duke	1932	4,000 ft.	Entebbe	*Damba*	*palpalis*	417	32	7·6	...

G. palpalis, which is not its normal vector) than is *T. gambiense*. Unless the data are entirely misleading, it follows, therefore, that the highest degree of adjustment between the polymorphic trypanosomes and tsetse will be found in *morsitans* and *pallidipes* areas. For this reason, the records set forth in the Tables are of interest, and for this reason also it is very desirable to obtain further reliable observations about these two species of tsetse in nature.

It is perhaps not out of place to record here some observations that illustrate the way in which tsetse and the trypanosomes they carry depend upon certain species of animals. Along the Ngussi River in the Northern Province of Uganda, when I visited the area in 1914, game was very plentiful. Kob, water-buck, elephant, hippo and especially buffalo were all seen close to the river. *G. pallidipes* was common, and a few *G. brevipalpis* and many *G. palpalis* were caught in the narrow strip of forest running along the river's edge. Of 65 *G. palpalis* dissected, 3 per cent contained gland-infections—a very unusual number in any sample of wild tsetse. Ninety-five *G. palpalis* were dissected, and of these 8·4 per cent. contained flagellates, 2·2 per cent. proboscis-only, 1 per cent. gut-only, and 5·2 per cent. proboscis-and-gut. The proportion of *T. congolense* infections is unusually high for *G. palpalis* in Uganda. No *T. grayi* was found in any of the three species of fly.

When I revisited my old camp in 1932, there was hardly a head of game to be seen, save hippo which were as numerous as ever. The rinderpest of a year or two ago had wiped out the buffalo (day-old tracks of a single animal only were seen), and in many hours' hunting we only saw occasional signs of kob and water-buck. Even elephant were rare visitors. During an 8-days' stay at the river, 5 *pallidipes* were caught and dissected and none contained flagellates. This species of fly, numerous in 1914, had apparently all but disappeared with the buffalo and other ruminant game. On the first day, two hippo were killed for food for the porters, and the remainder of the school moved off far up stream and were never seen or heard again near the camp.

The daily catches of *G. brevipalpis* were as follows :—

1st day 1♂
2nd day 1♂
3rd day 6♂♂ 3♀♀
4th day 30♂♂ 15♀♀
5th day 24♂♂ 13♀♀
6th day 17♂♂ 11♀♀

This large species is known to depend mainly on hippo and elephant, and in the present instance, on the departure of the hippo, these flies were constrained to reveal themselves in search of food. Only one of the 121 *G. brevipalpis* dissected contained flagellates—a gut infection of a mammalian trypanosome. The *palpalis* dissections are also of interest. Six hundred and seventy males and 259 females were dissected. Of these 929 flies, 10 (1 per cent.) showed infections of gut and proboscis, and 8 (0·8 per cent), gut-only infections. Again no *T. grayi* was found. There are no crocodiles along these reaches of the Ngussi, but a few *Varanus* frequent the river banks.

The few specimens of proboscis-and-gut infections in these later Ngussi *G. palpalis* that were stained and examined corresponded with the description given by Lloyd and Johnson for *T. congolense*. The absence of proboscis-only infections is very striking, as this is usually the commonest kind in any sample of wild flies that have access to game. The fall in the infection rate of *G. palpalis* is striking, and must be ascribed to the disappearance of ruminant game. The *palpalis* were undoubtedly feeding freely on the numerous hippo before our arrival frightened the animals away, and it is possible that *T. congolense* may occur in hippopotamus in nature. On the other hand, the rarity of flagellates in the *G. brevipalpis* dissected makes this unlikely, as this big species of tsetse undoubtedly depends very largely for its food on hippo and on elephant. It is more likely that the proboscis-and-gut infections in the *G. palpalis* were acquired from antelope, especially bush-buck, which still pass occasionally through the forest belt, and the evidence we have been considering tends to show that the hippopotamus does not act as a host of *T. congolense* in the Ngussi area.

Another interesting observation was made later in the same safari, at Fajao, below the Murchison Falls on the Victoria Nile. In 1914, antelope, buffalo and elephant were exceedingly numerous, the country being a closed and uninhabited sleeping sickness area. The Nile in this region swarms with hippo and crocodiles. In 1932, the game was found to be enormously reduced in numbers, presumably by rinderpest. The neighbourhood of Fajao has always been a great buffalo country, and I met with many herds of these animals there in 1914. In 1932, in the course of long detours inland, I saw no signs of buffalo. There were elephant and, near the river, a few water-buck and kob, and *G. morsitans* was certainly much less numerous than in 1914. Baboons were as common as ever, and no doubt serve as food for the tsetse. Hippo from the river roam for several miles inland during the night, and are frequently to be seen feeding ashore in daylight. *G. morsitans* was found in several restricted localities a mile or two from the river, and a few specimens of this fly followed me on several occasions back to the water's edge. All along the banks of the Victoria Nile are innumerable crocodiles. In 1914, no signs of *T. grayi* were detected in any of the *G. morsitans* then dissected. In 1932, 3·8 per cent. of the wild *G. morsitans* examined were carrying this trypanosome. *G. morsitans* has not hitherto been known to attack reptiles in nature, and has never been found to carry *T. grayi*. In order, therefore, to confirm the observation, I sent slides of some of the infected flies to Dr. Wenyon, of the Wellcome Research Bureau. Dr. Wenyon kindly sent me a report on these slides by Dr. Hoare, stating that the flagellates were of the *grayi* type, and that, although *T. ingens* or an allied form could not definitely be excluded, there was little doubt that they were indeed *T. grayi*. There is, therefore, no reasonable doubt that *G. morsitans* will on occasion feed on reptiles, and that in the present instance the scarcity of game has led to the fly's resorting to the crocodiles along the river bank.

There is one more point which it will be well to note before leaving this topic. Lloyd and Johnson in Nigeria never found *T. grayi* in *G. morsitans*, and they showed that the number of infections with pathogenic mammalian trypanosomes was much greater in *G. morsitans* than in *G. tachinoides*. Their figures also show, however, that if infections with *T. grayi* be included, the total percentage of trypanosome-containing flies is very nearly the same for both *G. morsitans* and *G. tachinoides*. My figures for the Fajao *G. morsitans* tell very much the same story. The percentage of *G. morsitans* carrying exclusively mammalian trypanosomes in 1914 was 9·6 per cent., the percentage in 1932, carrying a mixture of reptile and mammalian, 11·9 per cent. These facts, taken in conjunction with the observations made above on the subject of double infections, afford yet further evidence in support of the contention there advanced, namely, that in a certain proportion of any fly population trypanosomes can develop, whereas in many they cannot. According to this view, we may expect to find sooner or later among these Fajao *morsitans* double infections of the same individual fly with a reptilian and a mammalian trypanosome. Further, it would appear from the evidence that the trypanophil quality resides in the whole digestive tract and is not restricted to the gut or to the proboscis, and that it is beneficial to any of the species of trypanosomes that use tsetse in nature.

V. GENERAL INFERENCES

1. A consideration of the results of the dissection of wild tsetse by a number of observers in different parts of Africa suggests that the adjustment between the mammalian trypanosomes and the tsetse upon which they depend in nature is not very close. Of the pathogenic mammalian trypanosomes, the proboscis-only group, comprising *T. vivax* and its allies, is almost always the most widely distributed in wild tsetse, and the polymorphic group is the least successful.

2. In theory, transmission experiments with laboratory-bred tsetse should yield a higher infection rate than that obtaining in wild flies. Table II shows that the carrying power of the tsetse, under what appear to be abnormally favourable conditions, rarely exceeds 20 per cent. when an adequate sample of flies is taken.

3. There are indications that the full carrying capacity of a given tsetse population in nature may often remain untested owing to the prevalence in the area of food animals that are unsuitable hosts for the trypanosomes that depend upon the tsetse. Baboons and large reptiles, possibly elephants and probably hippo and wild pig fall into this category of food animals.

4. There are indications that only a certain limited number of individuals of any tsetse population are able to act as hosts for trypanosomes. The existence of double infections of the same fly with two different species of trypanosome supports this conclusion. These gifted individuals appear to constitute but a small proportion of a fly population and to be suited to all species of trypanosomes that can develop in tsetse, whether mammalian or reptilian. It is probable that species and also strains of trypanosomes differ in their ability to utilise these potential carriers.

5. The indications in favour of climate being an important factor in determining the infection rate are not at all clear. More experimental and field work is required on this subject.

6. There are indications that *G. pallidipes* may in certain circumstances attain a relatively high standard of efficiency as a carrier of the pathogenic mammalian trypanosomes. Whether this is determined by climate, or by the racial peculiarities or the feeding proclivities of this fly, cannot yet be determined.

7. Unilateral infections of the salivary glands have been shown to occur in both *G. morsitans* and *G. palpalis* in flies dissected during laboratory transmission experiments.

8. It has been shown that *G. morsitans* in nature may be infected with a trypanosome of the *T. grayi* type, an observation which strongly suggests that this species of tsetse on occasion feeds on reptiles in nature.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

II. ON THE TRANSMITTING POWER OF DIFFERENT RACES OF *GLOSSINA*

BY

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(*Received for publication* 25 *January*, 1933)

In a paper published in 1930, it was stated that experiments were being devised to find out whether different races of tsetse from different localities differ appreciably in their susceptibility to infection with trypanosomes (Duke, 1930a). Huff's work with *Culex pipiens* and *P. cathemerium* shows that, by selective breeding, races of the mosquito can be produced which transmit much more readily the parasite of bird malaria than do normal races. In the mosquito, only the female can serve as a vector, whereas in the tsetse both sexes are apparently almost equally efficient (Duke, 1930). Roubaud, years ago, suggested that geographical races of *Glossina* might occur which possessed greater transmitting power than others (Roubaud, 1920). In 1912, Kleine and Eckard carried out experiments to see whether tsetse developed any immunity to trypanosomes. They examined the offspring of flies that had been fed for some three weeks alternately on monkeys infected with sleeping sickness and on goats carrying *T. congolense.* The pupae deposited by these flies were collected, and the 434 flies which subsequently emerged were fed on infected monkeys. On the eighteenth day, the 402 survivors were dissected, and 11, or 2·7 per cent., were found to be infected. In another series of 90 flies, the experiment was continued for a month, and then the flies were fed in batches on 3 different monkeys: all 3 monkeys became infected, showing that at least 3 infective flies were present. These figures corresponded to those obtained under similar experimental conditions with young *Glossina*, whose parents had not been fed upon infected animals in the manner described. The authors concluded that there is no reason to believe that there are developed, in the body of a fly which daily takes up trypanosomes, anti-bodies which are transmitted to the next generation. In the experiments now to be described, two collections of pupae were obtained, one from Damba Island, Lake Victoria, and the other from the mainland shore line near Entebbe. The island of Damba has been uninhabited since 1909, and was declared a game reserve in 1926. It is heavily forested from end to end, and is remarkable for the large numbers of situtunga antelope that live upon it. At the present time, the animals, though still numerous, are less so than they were during the period 1910-25. There are also numerous crocodiles, and *Varanus*, and *G. palpalis* is very plentiful. The beach where the pupae were collected is about 20 miles, as the crow flies, from the mainland beach where the other collection was made. For the purposes of this experiment, we may safely assume that the Damba flies are completely isolated from the mainland; that they feed largely on antelope which carry both the polymorphic and the *vivax*-groups of trypanosome; and that, in years gone by, their ancestors acted as very efficient transmitters of sleeping sickness. The mainland beach that supplied the other collection of pupae lies a few miles from Entebbe, at a spot where there are practically no antelope, but plenty of hippo and crocodile. It is improbable that these flies in nature ever pick up any trypanosome save *T. grayi*. There are villages quite near by, the inhabitants of which during the last twenty years at least have been entirely free from sleeping sickness. Although the distance between the two points of collection of pupae is actually small, the degree of separation of the fly of the two areas is complete for all practical purposes; and, whereas the dietary of the fly on Damba includes trypanosome-infected blood, the only trypanosome ever taken up by the mainland fly is, in all reasonable probability, *T. grayi*.

METHOD

Pairs of boxes, each box containing originally some 50 flies hatched from one of the two collections of pupae, were fed twice, with a day's starvation in between the feeds, on a monkey carrying a trypanosome known to be transmissible by *G. palpalis*. *T. gambiense* or *T. rhodesiense* were the trypanosomes used. Flies dying during the first day or two of each experiment were, as usual, ignored. Thereafter all flies in the boxes were dissected as they died, and after a suitable time, usually 30 days, the survivors were killed with chloroform and dissected.

The details of the actual experiments need not be given in full. The results of the investigation were as follows :—

Mainland flies : Nakiaga.

Total dissected	1,066
Total containing flagellates	32
Percentage infected	3·0%

Damba flies.

Total dissected	1,114
Total containing flagellates	34
Percentage infected	2·9%

—a very striking agreement between the two groups of flies.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

III. *GLOSSINA MORSITANS*

versus

GLOSSINA PALPALIS AS A TRANSMITTER OF THE POLYMORPHIC GROUP OF TRYPANOSOMES

BY

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(*Received for publication* 25 *January*, 1933)

In 1912-13, Kleine and Fischer, near Rutschugi, six hours' march north of the great caravan route between Tabora and Udjiji, carried out a series of experiments to compare the transmitting power of *G. morsitans* and *G. palpalis*. Laboratory-bred flies were employed and a number of different strains of *T. gambiense*. Paired boxes, each component containing originally the same number of flies, were fed either simultaneously or one immediately after the other on monkeys (*Cercopithecus rufov.*) infected with this trypanosome. It is not quite clear whether every fly that died during these experiments was dissected, though from the text this appears to have been done. Experiments where infection of a clean animal did not occur were continued for 70 days. At the end of an experiment, all surviving flies were dissected and a preparation of the gut contents was examined for flagellates. Gland dissections were not made (Kleine and Fischer, 1913).

In these admirably conceived and executed experiments, 881 *G. palpalis* and 881 *G. morsitans* were employed. Twenty-three *G. palpalis* and 54 *G. morsitans* developed infections, i.e. 2·6 per cent. and 6·1 per cent. respectively. Commenting on these figures, the authors remark that the mortality of *G. palpalis* after the thirtieth day of the cycle exceeded that of *G. morsitans* by 15 per cent. Up to that date numbers remained approximately the same. They consider that this factor may partly explain the difference noted in the behaviour of the two species. In their concluding remarks they state that the connection between sleeping sickness and *G. palpalis* is not determined by the peculiar 'specificity' of this fly for this species of trypanosome, but by the climatic characters of the lake and river areas where sleeping sickness is normally found in Africa, an environment especially favourable to the development of *T. gambiense*.

EXPERIMENTAL

We will now consider the experiments performed during the latter part of 1932 at Entebbe.

The *G. morsitans* used in these experiments were derived from pupae sent to Entebbe through the kindness of Mr. C. F. R. Swynnerton and Dr. C. H. N. Jackson, of the Tsetse Reclamation Department, Tanganyika Territory. The pupae came by air, and reached Entebbe about 24 hours after their despatch from Dodoma.

The experiments are divided arbitrarily into 3 series, corresponding to the different consignments of pupae received from Dr. Jackson. The data will be presented in 3 groups, one for each series of experiments.

SERIES I

Infecting animal	Species of trypanosome	*G. morsitans*		*G. palpalis*	
		Number dissected	Number +	Number dissected	Number +
M. 989	*T. brucei* (1)	31	7	48	0
M. 974	*T. gambiense** (Str. Brander)	27	6	42	0
M. 983	*T. brucei* (2)	41	5	42	3
M. 981	*T. brucei* (3)	70	4	100	2
M. 996	Hornby vir. *brucei*	125	4	145	1
M. 985	*T. brucei* (1)	135	5	153	4
Reedbuck	*T. rhodesiense* (1)	44	9	53	1
M. 984	Hornby vir. *brucei*	166	14	193	2
M. 991	*T. rhodesiense* (2)	37	4	53	2
	TOTAL ...	676 i.e.	58 8·5%	829 i.e.	15 1·8%

* Indicates a strain no longer capable of infecting the salivary glands of *G. palpalis*.

SERIES II

Infecting animal	Species of trypanosome	*G. morsitans*		*G. palpalis*	
		Number dissected	Number +	Number dissected	Number +
M.974	*T. gambiense** (Str. Brander)	290	3	292	7
Sheep 51	*T. brucei* (1)	125	8	133	5
M.943	*T. brucei* (3)*	174	0	157	2
M.969	*T. brucei* (1)	147	16	179	10
M.901	Hornby vir *T. brucei*	245	19	279	5
M.977	Hornby vir *T. brucei*	142	7	68	6
	TOTAL	1,123 i.e.	53 4·7%	1,108 i.e.	35 3·1%

* Indicates a strain no longer capable of infecting the salivary glands of *G. palpalis*

SERIES III

Infecting animal	Species of trypanosome	*G. morsitans*		*G. papalis*	
		Number dissected	Number +	Number dissected	Number +
Sheep 11	*T. brucei* (1)	109	9	53	3
Sheep 27	*Damba*	33	5	35	5
M.924	*T. rhodesiense* (4)	57	1	55	3
M.923	*T. rhodesiense* (3)	21		27	2
M.954	*T. rhodesiense* (5)	41	4	38	2
M.963	*T. rhodesiense* (2)	118	4	135	7
M.962	*T. rhodesiense* (1)	60	3	65	0
	TOTAL .	439 i.e.	38 8·6%	418 i.e.	22 5·2%

The totals for the three series of experiments are:

G. morsitans.	*G. palpalis.*
2,238 flies dissected.	2,355 flies dissected.
149 flies contained flagellates.	72 flies contained flagellates.
i.e. 6·6 per cent.	i.e. 3 per cent.

The grand total for all the experiments discussed in this paper, including those of Kleine and Fischer, is therefore :—

G. morsitans.	*G. palpalis.*
3,119 dissected.	3,236 dissected.
203 positive.	95 positive.
i.e. 6·5 per cent.	i.e. 2·9 per cent.

The figures in each of these series were now examined in time by the formula $\chi^2 = \int \frac{(x-m)^2}{m}$ and the values so obtained for χ^2 in each of these series were as follows:

Series I = 36·950.
Series II = 3·582.
Series III = 3·822.
Series K & F = 13·050.

Reference to statistical tables demonstrates the real significance of the values of χ^2 for Series I and III and for Kleine and Fischer's experiment. In Series II, the result is on the borderline of significance.

The four series treated as a whole give a value of χ^2 of 45·28, testifying even more strongly to the significance of the results obtained.

In considering these figures, it must be remembered that several of the strains of trypanosomes used were either non-transmissible or very feebly so. At the outset, one of the objects of the investigations was to see whether a strain non-transmissible by *G. palpalis* would be transmissible by *G. morsitans*, and to settle this point it was desirable to employ strains of this nature. It is possible that, by employing readily transmissible strains throughout, the difference between the two species of tsetse would have been still more pronounced.

NOTE ON THE TEMPERATURE CONDITIONS UNDER WHICH TRANSMISSION EXPERIMENTS ARE CARRIED OUT AT ENTEBBE

Full details of the daily wet and dry bulb records at the actual spot in the laboratory where the fly boxes are kept will be given in a later paper in this series. Readings are taken at 8 a.m., 12.20 p.m. and 4 p.m. Dry bulb readings vary between 67° and 93° F. The mean dry bulb monthly readings vary from a minimum of 70·3 for September and a maximum of 78·2 for February. The mean humidity was lowest in December (70·4 per cent.), and highest in March (81·6 per cent.). There is thus little variation through the year. It is rare for the temperature to rise above 80° F. at the flies' resting place in the laboratory during the day. After closure of the laboratory for the night the temperature rises several degrees, 94° F. being the highest recorded temperature.

DISCUSSION

Examined by the formula that we have applied to the Entebbe figures, Kleine and Fischer's results show for χ^2 the value of 13·050, which testifies strongly to their genuine significance.

With considerable hesitation I venture, therefore, to disagree with the inferences drawn by these experienced observers from the results of their experiments. Their results agree with my own, and all the 3 series of experiments described in this paper agree with one another in the tale they tell.

The authors evidently believe that the difference between the figures for *G. morsitans* and *G. palpalis* in their experiments is due, partly to the higher mortality of *G. palpalis*, and partly to some unknown factor, climatic in nature, which placed *T. gambiense* at a local disadvantage in this its normal vector. The reason for this conclusion is apparently that in their experience, in regions where the climate favours the development of *T. gambiense*, from 2·5 to 6 per cent. of the flies used in transmission experiments became infected. In the light of more recent work on the transmissibility of trypanosomes by tsetse, this generalisation cannot be allowed as evidence ; and actually the authors' own data show that 2·6 per cent. of their *G. palpalis* were infected with trypanosomes.

The conclusion thus seems inevitable that, *ceteris paribus*, *G. morsitans* is a more efficient vector of the polymorphic trypanosomes than is *G. palpalis*; or, more accurately expressed, the *G. morsitans* of Tanganyika Territory is a better vector than is the *G. palpalis* of Lake Victoria.

There are one or two points about the experiments themselves that merit attention. *T. gambiense*, strain Brander, which I brought out to Entebbe from Professor J. G. Thomson's laboratory at the London School of Tropical Medicine and Hygiene at the end of 1931, has been very thoroughly tested with *G. palpalis*. Several thousand flies have been employed, and, although a number of heavy gut-only infections have been recorded, the strain has proved non-transmissible, both at ordinary laboratory temperatures (67° to 93° F.) and in the hot room at 90° to 92° F. This same strain was tested with *G. morsitans* and the results were practically identical. *G. morsitans* provided a slightly larger percentage of infected flies than *G. palpalis*, but no gland infections occurred. Thus the greater efficiency of *G. morsitans* did not enable it to overcome the inability of this strain to infect the glands, a disability which had been already satisfactorily demonstrated by experiments with *G. palpalis*, and similarly with Hornby's virulent strain of *T. brucei*, which Corson found readily transmissible at Tinde by *G. morsitans* and possibly by *G. pallidipes* (Corson, 1932). This latter strain has been kept up by syringe passage in various laboratory animals for some 5 years, since its first isolation from an ox that had passed through and been infected in a fly-belt. Of several thousand laboratory-bred *G. palpalis* employed to test this strain at Entebbe, only one or two have shown a gland infection (the gland flagellates being on each occasion very scanty), although a number of others acquired heavy gut infections. With *G. morsitans* the percentage of gut-only infections was greater, but the few flies whose glands contained trypanosomes showed also very scanty infections of these organs. Here again the two species of flies behave similarly, except for the slightly increased susceptibility we have already noted in the game tsetse. It is hoped to carry the comparison still further, as the information to be acquired by parallel tests is of indisputable value in revealing the true rôle of trypanosome and of tsetse in the processes we are studying in this series of papers.

The *morsitans* pupa-grounds in Tanganyika Territory can supply all the requirements of both Dr. Corson and the Institute at Entebbe, provided that the supply is rendered accessible and is distributed in a manner consistent with our respective requirements. It is highly desirable that in the near future the need for an economical distribution of this valuable material be recognised by the authorities, and that facilities be provided whereby Dr. Corson and ourselves may benefit more fully than has been possible hitherto.

I am greatly indebted to Mr. Swynnerton and to Dr. Jackson for their kind co-operation in this matter during Dr. Corson's absence on leave. With his return to Tinde and Dr. Jackson's departure on leave, the situation requires reconsideration.

One of the outstanding questions in trypanosomiasis research appears to me to be the careful comparative study of the transmitting power of different species of tsetse, using both readily transmissible strains and strains of trypanosomes known to be non-transmissible by one species of *Glossina*. For this purpose the Institute needs *G. morsitans* pupae, in exchange for which, we can, if necessary, supply pupae of *G. palpalis*. The superior qualities of *G. morsitans* as a transmitter of polymorphic trypanosomes makes it also desirable to employ this species, together with *G. palpalis*, in the investigation at present in progress at Entebbe into the rôle of antelope as a reservoir for *T. rhodesiense*. The importance of these matters is, of course, relative. Sleeping sickness no longer exerts the appeal it did a few years ago, when conferences and commissions were the order of the day. But it must not be forgotten that for a number of years the British Government has maintained continuous research into the still unsolved problems presented by this disease, that money has been spent thereon, and that the investigations are gradually drawing near completion. In the interests of economy alone, it is worth while ensuring a successful issue, and the time is past when matters can be left entirely to the unaided initiative and effort of the investigator on the spot.

CONCLUSIONS

1. Evidence has been produced to show that *G. morsitans* is a more efficient vector of the trypanosomes of the polymorphic group than is *G. palpalis*.

2. Of a total of 3,119 *G. morsitans*, 203 (6·5 per cent.) were found on dissection to contain flagellates. Of a total of 3,236 *G. palpalis* treated in exactly the same manner, 95 (2·9 per cent.) contained flagellates. Of these grand totals, 881 flies of each species belonged to the experiments of Kleine and Fischer.

3. Although the carrying power of *G. morsitans*, as revealed by these experiments, appears to be almost twice as great as that of *G. palpalis*, even the former species, in the conditions of experiment prevailing at Entebbe, is but poorly adjusted to the requirements of the polymorphic group.

4. A strain of *T. gambiense*, judged to be non-transmissible by *G. palpalis* as the result of testing with several thousand flies, when tested by *G. morsitans* proved also non-transmissible by this species.

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ON A NEW NEMATODE, *PARAPHARYNGODON MAPLESTONI* GEN. NOV., SP. NOV., FROM A BURMESE LIZARD

BY

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(*Received for publication* 13 *February*, 1933)

On two occasions, a large number of nematodes were found in the intestine of the common Burmese lizard, *Calotes versicolar* (Daudin, 1802), the parasites obtained from the first being mature females only, whereas those from the second were both males and immature females.

ADULTS. Small. Lips three, simple, without papillae. Cuticle with coarse transverse striations. Oesophagus straight, ending in a rounded bulb demarcated from rest of organ by a slight constriction, containing a valvular apparatus. Lateral alae present only in male.

MALE. Length, 1·98 to 2·6 mm.; maximum thickness, 0·22 mm. Lateral alae extend from behind oesophageal bulb to a little anterior to cloaca. Oesophagus, including posterior bulb, 0·45 to 0·54 mm. long. Diameter of bulb, 0·09 to 0·11 mm. Nerve ring at approximately 0·11 mm. from anterior end of worm.

Caudal end curved ventrally, tail tapering gradually to a fine point. Cloacal aperture on a conical prominence, on anterior surface of which lies a pair of papillae. A second pair of pre-anal papillae on each side of body lying laterally and posteriorly to first pair. A pair of caudal papillae posterior to cloaca, just at the base of tail. Besides these, a single median papillae present just behind cloaca. Caudal alae, absent. Only one spicule present, approximately 0·076 to 0·09 mm. in length. Accessory piece absent.

FEMALE. 3 to 5·2 mm. long; maximum thickness, 0·36 to 0·45 mm. Oesophagus, including posterior bulb, 0·83 to 1·03 mm. long. Diameter of bulb, 0·15 to 0·198 mm. Nerve ring situated approximately 0·13 to 0·152 mm. from anterior end of worm. Excretory

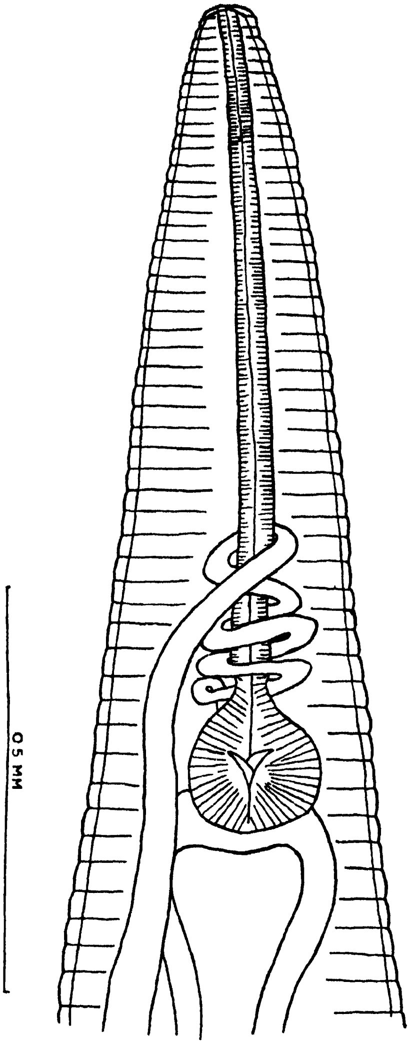

FIG. 1. *Parapharyngodon maplestoni* gen. n., sp. n. Anterior end of female, ventral view.

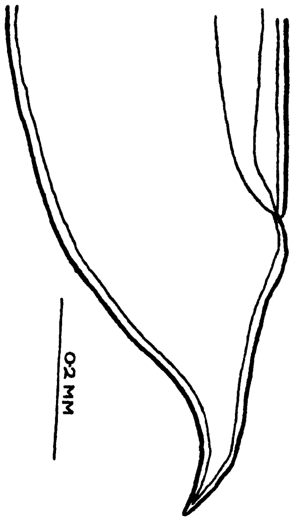

FIG. 4. *Parapharyngodon maplestoni* gen. n., sp. n. Posterior end of female, lateral view.

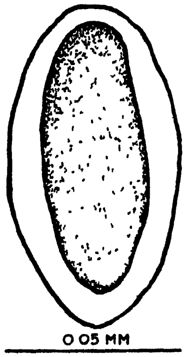

FIG. 5. *Parapharyngodon maplestoni* gen. n., sp. n. Egg.

pore in full-grown females approximately 1·35 mm. from anterior end. Tail, 0·31 to 0·4 mm. long, tapering for short distance behind anus. Vulva, with slightly projecting lips, near middle of body, but position variable, as in some specimens it may lie either a little anterior or a little posterior, the changes being probably due to varying degree of contraction and expansion during fixation. Vagina stout, muscular, running posteriorly, approximately 0·34 mm.

long; uteri convergent, passing into oviducts and ovaries. Coils of ovarian tubes anterior to oesophageal bulb. Eggs oval, 0·042 to 0·05 mm. by 0·08 to 0·091 mm.

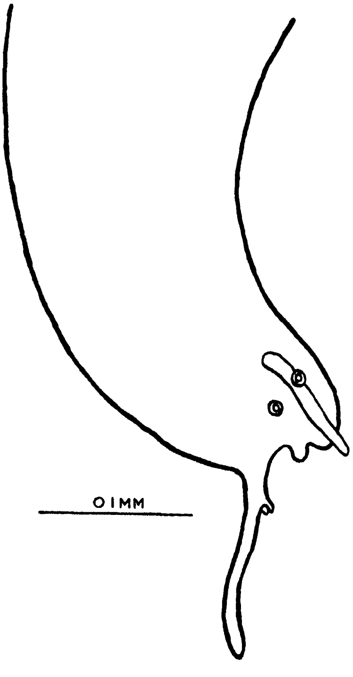

FIG. 2. *Parapharyngodon maplestoni* gen. n., sp. n.
Posterior end of male, lateral view.

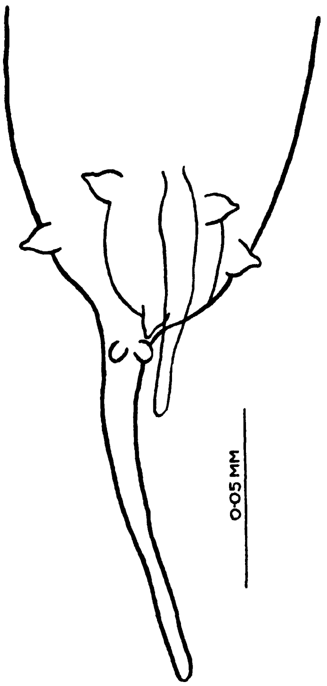

FIG. 3. *Parapharyngodon maplestoni* gen. n., sp. n.
Posterior end of male, ventral view.

This form appears to be closely related to the genus *Pharyngodon* Diesing, 1861, the chief differences being found in the absence of caudal alae in the male, in the forward position of the second pair of caudal papillae, the presence of an additional median unpaired papilla and a chitinised spicule, and the arrangement of the genital tubes in the female. Most of these characters being of generic importance, it is proposed to create for this form a new genus, for which the name *Parapharyngodon* is proposed, with *Parapharyngodon maplestoni* as the type, with the following diagnosis. Oxyurinae: mouth with three simple lips; cuticle with lateral flanges only in male; buccal capsule absent; oesophagus with posterior globular bulb containing a valvular apparatus; extremity of body truncate.

Male: caudal alae absent; caudal papillae consisting of two pairs pre-anal, one pair post-anal, and a median unpaired post-anal one; spicule single. Female: anus near end of body, a little anterior to commencement of the tapering tail; vulva near middle of body, with slightly projecting lips; eggs oval and elongate.

Type species: *P. maplestoni* gen.n., sp.n.

Host: *Calotes versicolar.*

Locality: Rangoon.

Type specimens have been deposited in the Helminthological Institute, Rangoon University.

Table of dimensions.

Parapharyngodon maplestoni gen. n., sp. n.

	Male	Female
	mm.	mm.
Length	1·98 to 2·6	3 to 5·2
Diameter, maximum	0·22	0·36 to 0·45
Oesophagus length, including bulb	0·45 to 0·54	0·83 to 1·03
Oesophageal bulb, diameter	0·09 to 0·11	0·15 to 0·198
Nerve ring from anterior end of body	0·11	0·13 to 0·152
Excretory pore from anterior end of body	...	1·35
Spicule, length	0·076 to 0·09	...
Approximate proportions into which vulva divides body length	...	1 : 1
Length of vagina	...	0·34
Anus to tip of tail	...	0·3 to 0·4
Eggs...	...	0·042 to 0·05 × 0·08 to 0·091

In conclusion, the author wishes to express his sincere thanks to Dr. P. A. Maplestone for the valuable help and advice rendered in the course of preparation of the present work.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE GENERA OF THE TRIBE MUSCINI, SUBFAMILY MUSCINAE, BASED ON A COMPARATIVE STUDY OF THE MALE TERMINALIA

I. THE GENUS *MUSCA* Linnaeus

BY

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INTRODUCTION

In an earlier paper (1932), I recorded the results of a comparative study of the male terminalia of practically all the species of the genus *Musca* known to me, and arrived at the following main conclusions :—

1. The species fall into three natural groups, the *domestica* containing the simplest forms, the *lusoria* the most advanced, while the species of the *sorbens* group occupy an intermediate position.

2. In each group there is one certain blood-sucking species, *planiceps* in the *domestica, crassirostris* in the *sorbens* and *inferior* in the *lusoria* group. The species *planiceps*, though possessing the most advanced larviparous habit, belongs to the simplest group. The remaining species exhibiting various phases in the evolution of the blood-drawing proboscis fall into the *sorbens* and *lusoria* groups. In other words, the blood-drawing proboscis has evolved independently at least once in each group, and in the two advanced ones more than once.

As this comparative study has not only resulted in the elucidation of the affinities and relationships of the species, but has also very materially helped in separating with certainty closely allied forms, I have decided to make a more extensive comparative study of these structures in other genera of the higher Diptera, in the hope of discovering their relationships to each other, re-defining the genera and establishing the identity of the various species. In the present paper I propose re-defining the generic characters in *Musca*, and emphasising the significance of the terminalic characters as a guide to the interpretation of those of allied genera. It is necessary, also, to take this opportunity of drawing attention to the structure of the proboscis in the blood-sucking species, for it still is very imperfectly understood, and to show how its structure confirms the terminalic findings.

But, before doing so, I would like to take this opportunity of sounding a note of warning, on the danger of drawing conclusions as to the identity and relationships of species and genera from incomplete studies of the male terminalia in the higher Diptera in general.

I believe erroneous conclusions may be arrived at for the following reasons :—

1. Imperfect understanding of the anatomy of the parts, and, following this, the failure to appreciate the true significance of the characters of the male terminalia in working out the identity and relationships of species, especially in closely allied forms.

2. Incomplete study of the parts. In some cases, only the characters of the anal cerci have been studied and used, in others only those of the phallosome, the characters of the ninth coxite and paramere being overlooked. It is most important that all these structures be studied together before any conclusions are drawn.

3. Study of the parts after drawing back the anal cerci, and exposing them and the phallosome for profile views, and illustrating these while still attached to the abdomen. It is quite true that many species, especially those of the Calliphorinae and Sarcophaginae, can be readily identified by exposing the anal cerci and the adjacent distal segments of the ninth coxite, which afford striking structural differences, so that anyone can use these characters for this specific purpose without further dissection and mutilation of the specimen. I have, however, extensive proof that it is in such preparations

impossible to study the structure and true relationships of the parts, especially those of the ninth tergo-sternum, the segments of the ninth coxite, the paramere and the phallosome, in order to arrive at the true affinity of the species. To demonstrate this, it is only necessary to compare the illustrations in Schnabl and Dziedzicki's (1911) work on the Anthomyidae, with illustrations of the parts treated with caustic potash dissected off and mounted without compression on a slide; their drawings are nothing more than caricatures of the originals.

4. The mounting of the parts flat on slides after treatment with caustic potash and dissection. The interpretation of the relationships of the parts in such mounts may be quite misleading, especially if illustrations of them are executed by artists who do not understand the anatomy of the male terminalia.

I maintain that the study of the male terminalia of the higher Diptera, as a guide to the relationships of species and genera and the separation of closely allied forms, can only be successfully carried out so as to afford reliable conclusions when the parts are dissected off, softened and relaxed in caustic potash, and cleared in clove oil or some other medium. The anatomy and relationships should then be studied in the oil in a suitable receptacle with the aid of dissecting needles, a binocular microscope and a good light. The parts should then, after dissection, be mounted separately on slides, without compression and always in one direction. Drawings of the parts should then be made with the same objective and eye-piece and with the aid of a camera lucida. The value of such studies is enhanced when the drawings are executed by the worker himself. Further, it is important to study the terminalia of large numbers of specimens of as many species as possible, and to compare them with those of allied genera. One is then in a position to appreciate the value of the characters of use in tracing out relationships and drawing sound conclusions.

This has been done in the studies of the male terminalia in *Musca* and many allied genera, and no conclusions have been drawn until illustrations of the parts of large numbers of species have been studied along with the preparations.

Tribe MUSCINI

Subfamily MUSCINAE

The subfamily Muscinae, as understood here, falls into two tribes, the Muscini and Anthomyini, the former representing the primitive stem and containing all the blood-sucking higher Diptera in three genera, *Musca*, *Stomoxys* and *Glossina*. These two somewhat natural divisions were recognised by Schnabl and Dziedzicki (1911), though much confusion is introduced into their work by the use of subfamily, group and form names, and unfortunately they began their study with some of the higher forms of the Anthomyini. Many genera now included in the Anthomyinae, or Anthomyidae, of authors belong to the tribe Muscini. It is not possible, as yet, to determine the exact limits of these two tribes, if such exists, until the male terminalia of many more species of Anthomyine genera have been studied from the comparative standpoint. In the tribe Muscini, the anal cerci are for the most part broad, and either triangular or quadrilateral in shape (wing-like), the two ends of the free margin varying in shape according to the species and genera. The basal segment of the ninth coxite is in the form of a lateral clasper, and the distal segment is small and not pincer-like in shape. There is one marked exception in this tribe, the species of the genus *Glossina*, in which the ninth coxite is almost entirely wanting, and the anal cerci in one group (*fusca*), at least, are completely separated and are claw-like. I will discuss the significance of these terminalic characters when dealing with *Glossina* in another paper.

GENUS *MUSCA* Linnaeus (emended Patton, 1933)

Generic Characters. Larva. Third stage larva with left oral hook smaller than right. Posterior spiracular plates somewhat D-shaped, with the usual three sinuous slits, the plate varying in size, shape and density according to the species; it is most dense and the slits are very narrow and long in most of the species of the *lusoria* group known at present. Third segment of antenna with a large grape-like sensorium, opening by a duct into the socket. Arista with simple spinulae almost to the tip on both sides, those on the upper side being more numerous and extending more towards the base. Proboscis retractile and completely concealed on ventral surface of head capsule when at rest. Sternopleural bristles three in number and arranged 1 : 2 (may be wanting). Vein M_{1+2} bends forwards elbow-fashion and ends close to vein R_{4+5} (and may even join it). Male Terminalia. Anal Cerci. Broad, triangular or quadrilateral in shape, united loosely along inner margins by a slip of chitin, and can be easily separated. Free margin varying in shape, the two ends tending to form nipple-like processes in the more advanced groups. NINTH COXITE. Proximal segment shaped like the human hand, minus fingers and thumb, directed towards the middle line and functioning as a lateral clasper of the ovipositor. Distal segment concealed, a small elongated hairy plate lying in the membrane at the outer margin of each anal cercus. NINTH TERGO-STERNUM. With rounded or square dorsal margin. PHALLOSOME. Uniform in structure throughout the genus, tubular in shape, partly chitinous and partly membraneous without any chitinous adornment (gadgets). POSTERIOR PROCESS OF PHALLOSOME. A well-developed process, present in all species, with concave face towards phallosome, either simple, or expanded distally, or forming a large, hood-like, commonly Y-forked expansion. APODEME OF PHALLOSOME. A curved narrow plate. PUMP SCLERITE OF EJACULATORY DUCT. Large and shaped somewhat like a lacrosse bat. PARAMERE. Structure varying according to the groups. In the *domestica* group, a simple plate consisting of two parts, the anterior longer, bearing several bristles, one usually very long; the posterior part a slightly raised, button-like plate with four, sometimes six, clear areas; in *sorbens* group the posterior part is raised to form a narrow, or wider, sugar-loaf process; in the *lusoria* group the posterior part is a long, raised, bent process. The two parts of the paramere are completely united to each other. FIFTH STERNUM. A large somewhat triangular plate with lateral ends produced to form long or short processes expanded distally and nearly always serrated.

NOTES ON THE GENERIC CHARACTERS. The small size of the left oral hook of the third stage larva is not strictly a generic character, as it is also small in the third stage larva of the species of the genus *Stomoxys* (sens. lat.). The basal, grape-like sensorium in the third antennal segment is, however, a most important generic character, for I have not found it in the antenna of the species of any other genus yet examined. The retractile proboscis is common to all the species and with other characters is important. Some notes on its structure and evolution in the blood-sucking forms is given further on. The wing-like shape of the anal cerci and their loose attachment to each other are important characters. The structure of the ninth coxite is also an important generic character. The structure of the phallosome, posterior process and the paramere afford a reliable guide not only to the species of the groups but also to the genus. The reader should consult my earlier paper (1932) for illustrations of the terminalia.

SIGNIFICANCE OF THE MALE TERMINALIA IN *Musca*. The male terminalia in *Musca* are an important starting point in the study of these parts in the other genera of the Muscini, for they undoubtedly represent a comparatively primitive type. The simple phallosome without any adornment, the simple paramere (especially in the *domestica* group), the functional, clasper-like proximal segment of the ninth coxite, the reduced distal segment, and the loosely joined, wing-like anal cerci are all structures which suggest an ancient type which has long become fixed. The fact that there are very few structural differences in these parts in the species further supports this view. In the higher genera of the tribe, the two parts of the paramere become separated and the phallosome develops various kinds of chitinous adornment; commonly the posterior process is wanting. These changes will be described in subsequent papers. Further, in the Anthomyini and the Calliphorinae, the distal segment of the ninth coxite becomes enlarged and modified in various ways, and in the latter, in particular, it comes to be closely associated with the anal cerci. Without the knowledge of the male terminalia in *Musca* and allied genera, it would be quite impossible to understand the corresponding structures in the Calliphorinae, Sarcophaginae, etc.

PROBOSCIS. As the structure of the proboscis, especially in the blood-sucking forms, is very imperfectly understood, it is necessary to refer briefly to it here, and to explain, with the aid of drawings, the probable line of its evolution. Another opportunity will be taken to deal more fully with this subject.

The genus *Philaematomyia* Austen was erected on the supposedly distinct proboscis of *crassirostris* from that of the genotype *domestica*. Malloch (1932) has recently resurrected this genus, and in doing so states that 'there are some species of Muscinae that have the proboscis with the apical section heavily chitinized and apparently fitted for piercing, or at least with some apical hooks attached to the rigid apical section which are adapted for abraiding the epidermis of mammals to the extent that blood may be produced from the wounds.' And, under the species *crassirostris*, he says: 'It is commonly found on cattle feeding on sores.' It is very necessary to correct these inaccurate statements, which are based on a misinterpretation of the structure of the proboscis, and especially that of the blood-drawing form, in *Musca*. This is the more necessary when it will be seen from the illustrations accompanying this paper that *crassirostris* is not the only species whose proboscis exhibits these changes. If this species is placed in the genus, then the six other species showing similar changes should also be placed in it. The structure of the proboscis of *crassirostris*, and the mechanism by which it draws blood, has been fully described by Cragg (1912);

his paper should be consulted for details. Here it is only necessary to point out that the apical section is not heavily chitinized but consists of the soft labella, which are quite well developed in all the blood-sucking forms, and cannot possibly pierce the skin, the only structures which first tear and then pierce the epidermis being the ends of the prestomal teeth. Further, there is no rigid apical section, the prestomal teeth (hooks of Malloch) being attached to the prestomal sclerite, which is freely movable. *M. crassirostris* does not feed on sores but on blood from a wound made in the skin. Malloch has evidently confused the feeding habits of *crassirostris* with those of such species as *M. bezzii*, *M. autumnalis*, *M. lusoria*, etc.

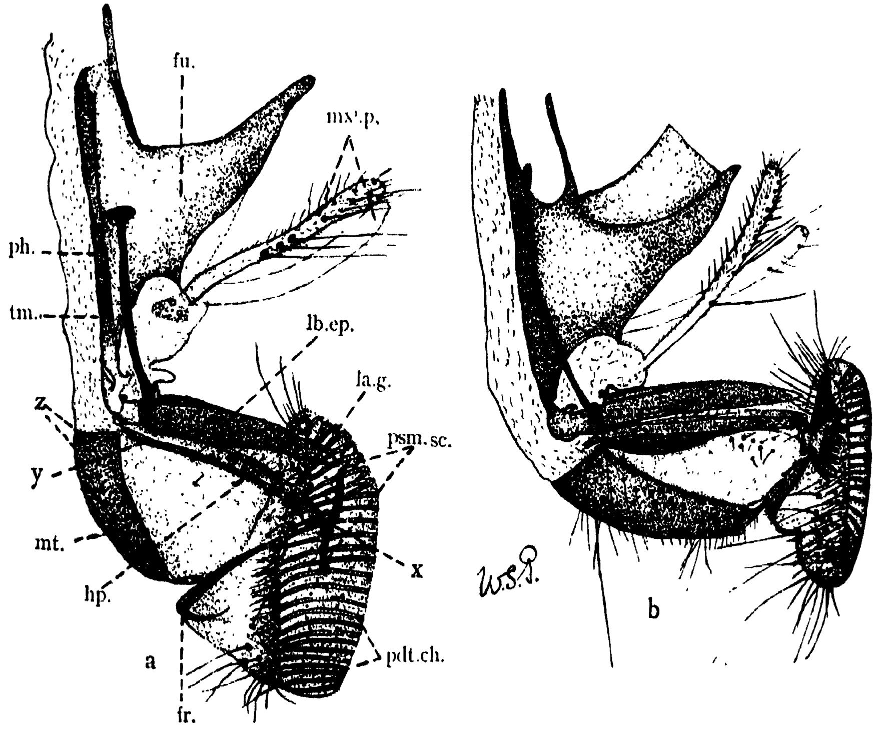

FIG. 1. *a.*—Proboscis of *M. domestica*, dissected off and mounted without compression on a slide. *fr.*—furca showing joint with mentum; *fu.*—fulcrum; *hp.*—hypopharynx; *la.g.*—labial gutter; *lb.ep.*—labrum-epipharynx; *mt.*—mentum; *mx'.p.*—1st maxillary palps; *pdt.cb.*—pseudotracheal channels; *ph.*—pharynx; *psm. sc.*—prestomal sclerite; *tm.*—torma; *x.*—position of prestomal teeth; *y.*—position of labial keel; *z.*—position of ridge of mentum. *b.*—Proboscis of *M. planiceps* mounted in same way for comparison (same scale).

EVOLUTION OF THE BLOOD-DRAWING PROBOSCIS IN *Musca*. At the present time there are seven species known to me which exhibit various phases in the evolution of the blood-drawing proboscis. On their terminalic characters they are grouped as follows :—

GROUP I (*domestica*). *Musca planiceps*. A voracious blood-sucker with an advanced larviparous habit. Widely distributed in the Oriental region.

GROUP II (*sorbens*). 1. *Musca conducens*. Can scratch off a scab but is unable to draw blood from the intact skin. Oviparous. Widely distributed in the Ethiopian, Oriental and Australasian regions. 2. *Musca crassirostris*. A voracious blood-sucker. Oviparous. Widely distributed in the Palaearctic, Ethiopian and Oriental regions.

GROUP III (*lusoria*). 1. *Musca mesopotamiensis*. Can scratch off a scab but is unable to draw blood from the intact skin. Oviparous. Palaearctic region. 2. *Musca fletcheri*. Probably a blood-sucker. Early stages unknown. India, Assam, and probably widely distributed in the Middle East. 3. *Musca seniorwhitei*. Probably a blood-sucker Early stages unknown. East side of India and Middle East. 4. *Musca inferior*. A voracious blood-sucker. Early stages unknown. Widely distributed in Oriental region.

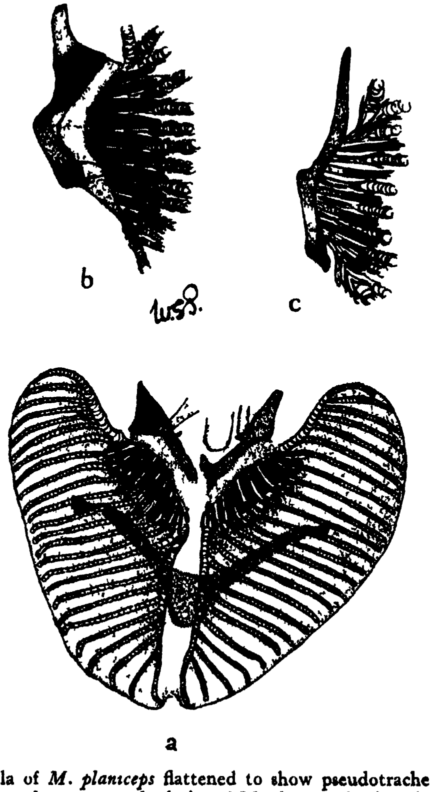

FIG. 2. *a*.—Labella of *M. planiceps* flattened to show pseudotracheal membrane, prestomal sclerite (split), teeth, etc.; *b*.—prestomal sclerite of *M. planiceps* in side view showing teeth, etc., of one half; *c*.—prestomal sclerite of *M. domestica* in side view for comparison (same scale).

Musca planiceps. As this species is closely related to *domestica* and allies, we would expect that the structure of its blood-drawing apparatus confirms this relationship, and this proves to be the case. The proboscis of *domestica* and that of *planiceps* are illustrated in fig. 1. The important structures are labelled in the drawing of the former so that the corresponding structures may be recognised in the other illustrations. Except for the changes noted, the two are structurally alike. The mentum of *planiceps* is longer and deeper, and has a well-developed ridge on its proximal end for muscle attachment; this is wanting in *domestica*. The labial gutter shows a well-developed, long keel occupying more than half of the posterior surface, also for muscle attachment; this is also wanting in *domestica*. The prestomal sclerite in *domestica* (fig. 2, *c*) is long and narrow, especially the dorsal free arms, and the axial apophysis projects downwards. The five teeth are slender rods serrated at their ends, and are attached directly on to the prestomal sclerite, there being no tooth plate. The interdental armature consists only of narrow rods, which have developed from the proximal rings of the pseudotracheae between the teeth. The prestomal sclerite in *planiceps* (fig. 2, *b*) is relatively shorter and wider, especially the dorsal free arms, and the part bearing the tooth plate is arched. The tooth plate is strongly developed and extends beyond the axial apophysis, which is slightly elongated, bluntly pointed, and projects upwards and forwards. The nine strong teeth (and often a small tenth) are stout blades, bluntly pointed and markedly serrated at their ends, which project freely from the pseudotracheal membrane. The labella are large, and there is only a slight reduction in the number of the pseudotracheae, there being about 25 to 26 in *planiceps*, whereas there are usually 28 to 32 in *domestica* (fig. 2, *a*).

The mechanism by which blood is drawn has been studied in specimens showing the teeth in action, by dissecting off the proboscis and clearing it in caustic potash. When the proboscis is applied to the skin, the labella being separated, the V-shaped prestomal sclerite is rotated in an antero-posterior direction on the pivot formed by its articulation with the rods of the labial gutter, and the large surfaces of the internal walls of the labella come to lie at right-angles to the labial gutter. The broad, dorsal, free arms of the prestomal sclerite diverge from each other, but are only slightly drawn back. The axial apophysis lies opposite the sixth or seventh tooth (counting from above), and, owing to the width of the dental plate, does not project beyond the ends of the teeth, and, therefore, takes no part in fixing the prestomal sclerite. The labella are now drawn back, the teeth project from the pseudotracheal membrane, and as the eversion continues they are drawn across the skin. Their free serrated ends, on these movements being rapidly repeated, tear through the skin and draw blood. The blood-drawing apparatus of *planiceps* then exhibits a simple advance on that of *domestica*. Four additional teeth have developed, and each tooth is a blunt, serrated, stout blade, unlike the rose-thorn teeth of *crassirostis*.

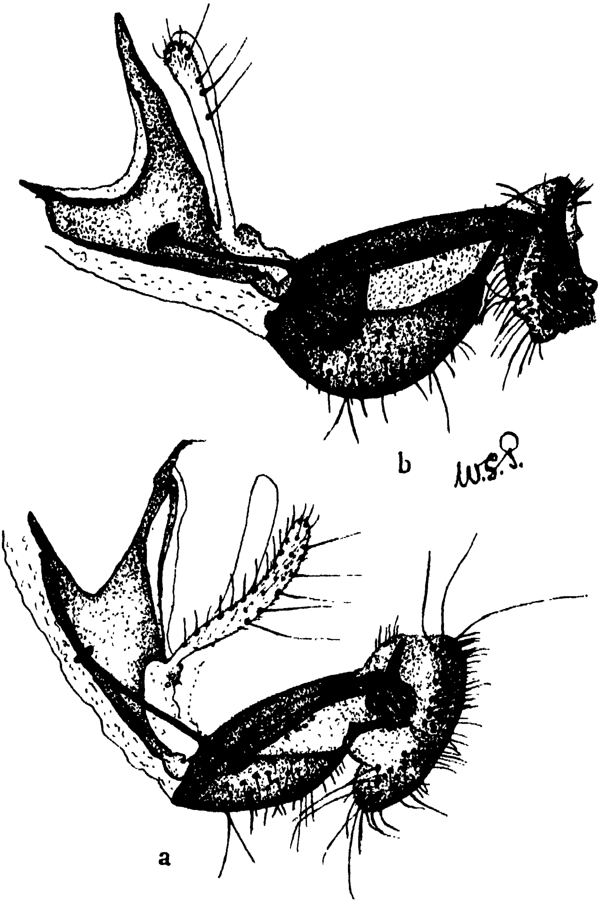

FIG. 3. *a.*—Proboscis of *M. conducens*; *b.*—same of *M. crassirostris*, with labella not quite in resting position (same scale).

The mentum, labial gutter and prestomal sclerite only exhibit slight changes for the functioning of the 18 comb-like teeth. The mechanism of the proboscis, in the act of drawing blood, is exactly similar to that of the proboscis of *domestica* when it scrapes the skin with its delicate teeth.

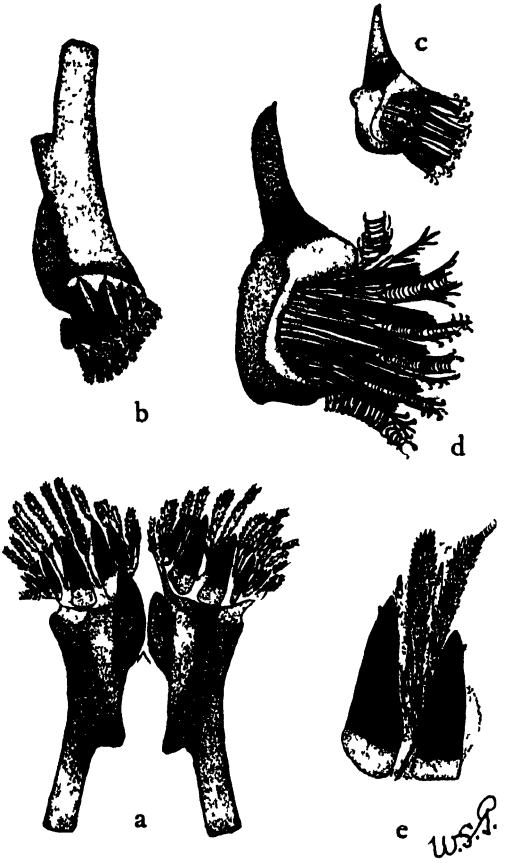

FIG. 4. *a.*—Prestomal sclerite of *M crassirostris*, split and spread out to show inner surfaces of two halves; *b.*—prestomal sclerite of *M. crassirostris* in side view, showing teeth, etc., of one half; *c*—prestomal sclerite of *M conducens* in side view, drawn to same scale as *b*; *d*—prestomal sclerite of same in side view, enlarged to show prestomal sclerite, teeth, axial apophysis, etc., *e*—two median teeth, serrated blades, and one pseudotracheal channel of *M. crassirostris* enlarged.

Musca conducens and *M. crasssirostris*. These two species are closely related, and we would expect that the blood-drawing apparatus shows evidences of this relationship, which a study of it confirms. The proboscis of each is illustrated in fig. 3. A comparison of these shows that, although there is a wide gap between the two, that of *conducens* represents an early phase towards the more completely developed tearing apparatus of *crassirostris*. The mentum of *conducens* is well developed, and has a small ridge on its posterior part for muscle attachment; the mentum of *crassirostris* is bulbous, heavily chitinised, and has a strong projecting ridge. The labial keel is developed in *conducens*, but is short and small (compare with that of *planiceps*, fig. 1, *b*), whereas in *crassirostris* it is a short, deep plate projecting into the hollow mentum. The prestomal sclerite in *conducens* (fig. 4, *c*, *d*) is short, the part carrying the dental plate and teeth is well arched, and the axial apophysis is relatively long, pointed, and directed forwards beyond the plate. There are five long, bluntly-pointed teeth, shaped like rose-thorns when seen in side view; the interdental armature consists of elongated rods on each side of the pseudotracheae, which have developed from the basal pseudotracheal rings; it is important to note that they are branched at their distal ends. Each half of the prestomal sclerite of *crassirostris* (fig. 4, *a*, *b*) is a strong, straight rod, the distal part elongated, the end arched and bearing the dental plate and teeth. The axial apophysis is strongly developed and is in the shape of a strong median tooth. The five teeth (one is usually small) are shaped like rose-thorns, and are firmly embedded on the dental plate close to each other (fig. 4, *a*, *b*). The median pair (fig. 4, *e*) are strongly developed, their apices bluntly pointed and serrated, and are inserted higher up than the two outer ones, owing to the arching of the tooth plate. Between the teeth, and extending distal to them, are the serrated blades (fig. 4, *e*), which are attached to a long rod inserted on the dental plate. Towards the end of the teeth the rod divides, and each part carries five or six blades consisting of deeply serrated slips of chitin arising at different levels and superimposed, so that in all there are five sets of blades, each bearing five or six serrated leaflets. These sets of blades diverge from each other in front of the teeth and bend upwards, superficial to the five pseudotracheae, which are much reduced in calibre. At the point of bifurcation of the rod there is a pair of leaf-like blades (fig. 4, *e*). From a comparison of the interdental armature of these two flies, it is suggested that the branched rods in *conducens* represent an early stage in the development of the serrated blades in *crassirostris*, for there can be little doubt from their position and relation to the pseudotracheae that they both have a common origin, namely, the proximal rings of the pseudotracheae. The axial apophysis in *conducens* shows evidences of developing into a pointed tooth-like structure, as in *crassirostris.*

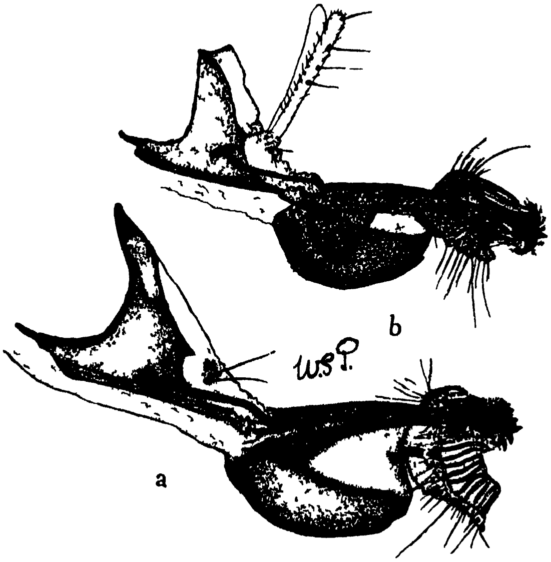

Fig 5 *a*—Proboscis of *M inferior* with prestomal sclerite in position when drawing blood, note everted teeth, and central axial apophysis with point extending beyond teeth, *b*—same of *M crassirostris*, also with prestomal sclerite in same position, note prestomal teeth and position of axial apophysis, both drawn to same scale

A study of the feeding habits of *conducens* leaves no doubt that it can only scratch off a scab and enlarge an already existing wound. The mechanism by which this is brought about is as follows :—When the proboscis is extended and applied to the skin, the Y- or U-shaped prestomal sclerite is drawn backwards, and the dorsal diverging arms form about half a right-angle with the labial gutter. The axial apophysis now projects forwards, the point extending well in front of the teeth, exactly as does the axial apophysis of *crassirostris* (fig. 5, *b*) ; it penetrates the skin and fixes the prestomal sclerite. The pseudotracheal membrane is now drawn back and the teeth are everted, and their free ends are seen directed outwards, forming a ring of claw-like points around the prestomum. These movements being rapidly repeated, the teeth are strong enough to scratch off a scab or enlarge an already existing wound.

The mechanism by which *crassirostris* can tear through the intact skin and draw blood is very similar. Owing to the structure of the prestomal sclerite, it is drawn right back into line with the labial gutter, and is now fixed by the axial apophysis (fig. 5, *b*); the labella, and with them the pseudotracheal membrane, are now drawn back, and the serrated blades and teeth are everted and swept across the skin, which is rapidly torn, and blood is drawn. The length of the prestomal sclerite, its rod-like shape, and the fact that it can be drawn right back in line with the labial gutter gives a long range of excursion for the powerful teeth. The blood-drawing apparatus in action in *crassirostris* is illustrated in fig. 5, *b*. Although there is a wide gap between the proboscis of *conducens* and that of *crassirostris*, both exhibit a common line of development. And these findings, therefore, confirm the conclusion reached, as a result of the study of the male terminalia, that they are closely related.

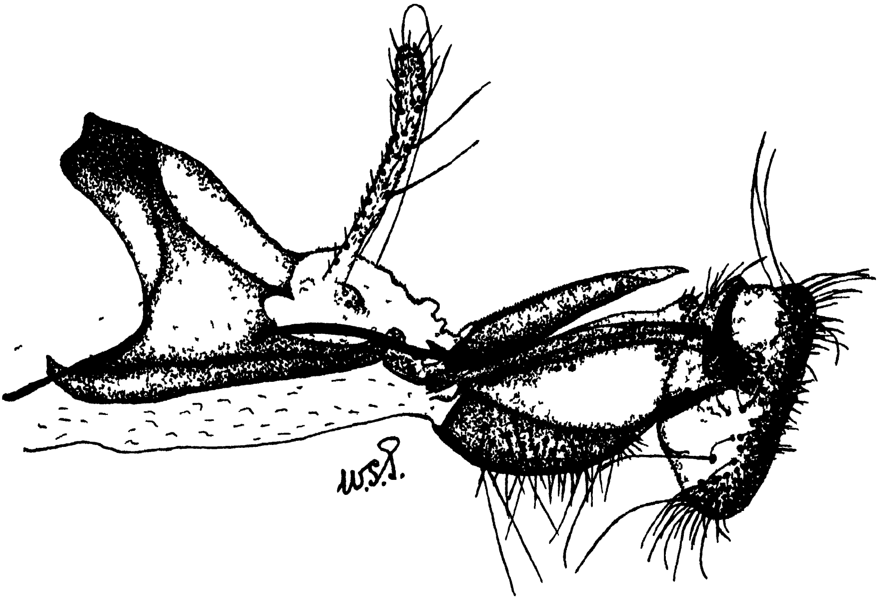

FIG 6 Proboscis of *M. mesopotamiensis*, note the small ridge on mentum and keel

The genus *Pristirhynchomyia* was erected by Brunetti (1910) for *conducens* (*lineata*), also on the supposedly distinct nature of its proboscis from that of *crassirostris*. Unfortunately, Brunetti misinterpreted the structure of the proboscis, actually reversing it. This error was corrected by Patton and Cragg (1912).

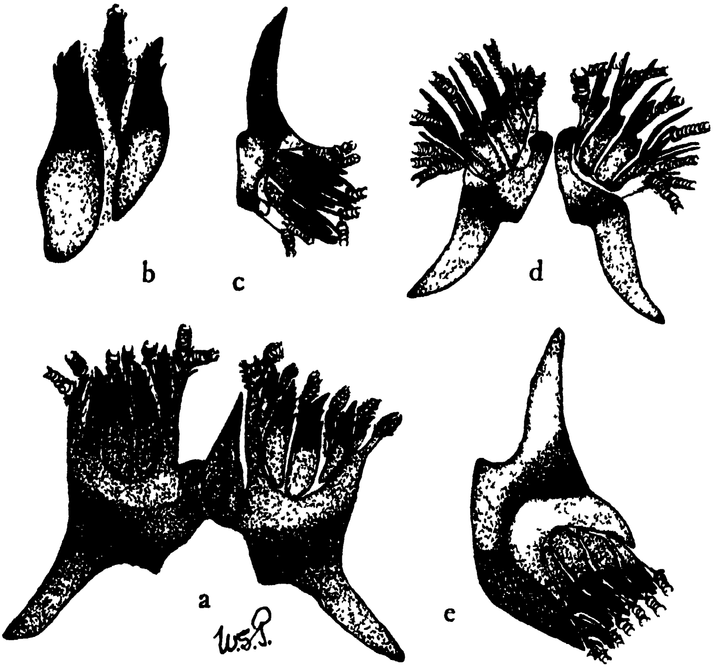

FIG. 7. *a.*—Prestomal sclerite of *M. fletcheri*, split and spread out to show inner surfaces of two halves; *b.*—median teeth of *M. fletcheri*, and commencing formation of interdental armature from proximal rings of pseudotrachea; *c.*—prestomal sclerite of *M. mesopotamiensis* in side view, showing teeth, etc., of one half; *d.*—prestomal sclerite of same, split and spread out to show inner surfaces of two halves (drawn to same scale as *a*); *e.*—prestomal sclerite of *M. fletcheri* in side view, to show teeth, etc., of one half (drawn to same scale as *c*).

Musca mesopotamiensis*, *M. fletcheri*, *M. senior-whitei and ***M. inferior***. On the characters of their male terminalia these four species are related to each other. *M. mesopotamiensis* exhibits an early phase in the evolution of the scratching proboscis in this series. Its proboscis is illustrated in fig. 6. The mentum is well developed and has a small posterior ridge for muscle attachment; the labial keel is small and short, and only projects a little. The prestomal sclerite has long, stout, pointed dorsal arms, the remaining part is short, and the axial apophysis small and rounded apically. There are four teeth, three of which are strongly developed. The interdental armature consists of long, *unbranched*, bluntly-pointed, narrow rods, developed from the basal rings of the pseudotracheae, of which there are five opening into the prestomum. Although this species has quite strongly developed teeth, it is unable to do more than scratch off a scab and perhaps enlarge a wound; the prestomal sclerite is illustrated in fig. 7, *c*, *d*. Its scratching armature is in much the same stage of development as is that of *conducens* in Group II. Its mechanism is much the same as in that species.

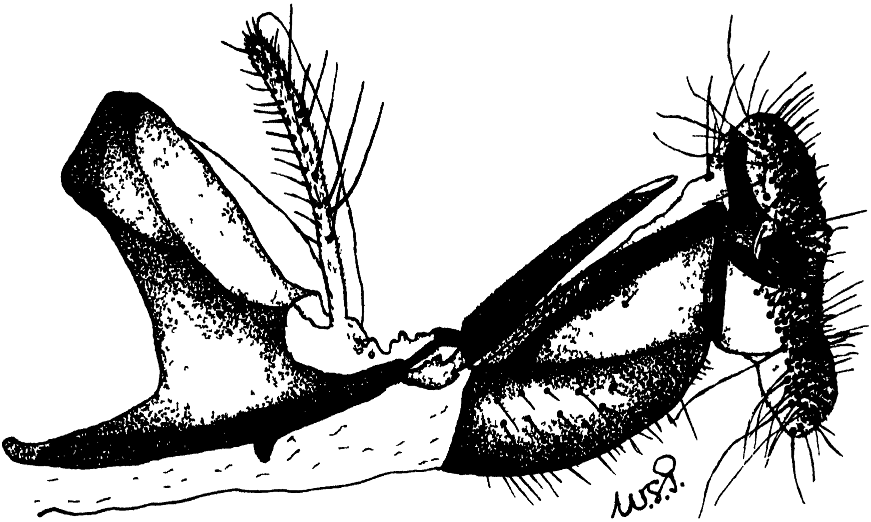

Fig. 8. Proboscis of *M. fletcheri*

The proboscis of *M. fletcheri* is illustrated in fig. 8. The mentum is long and deep and strongly chitinised, and the posterior ridge is well developed; the keel is short and projects slightly into the mentum. The prestomal sclerite (fig. 7, *a*, *e*) is short, and is very similar to that of *mesopotamiensis*; the dorsal arms are short and bluntly pointed. The remaining portion is short and deeply arched, bearing the wide, dental plate, and a large tooth-like axial apophysis which projects beyond the plate. There are five strongly serrated teeth, one usually smaller than the others. The interdental armature is poorly developed, the basal rings forming short rods. A comparison of its proboscis with that of *mesopotamiensis* and *inferior* suggests that it occupies an intermediate stage, the structure of the prestomal sclerite and teeth suggesting a close relationship to these species. Until recently I have only had the opportunity of studying the type material, consisting of a single male and female. Mr. Colbran J.

Wainwright recently gave me for study a large collection of no less than eight species of *Musca*, most of which were collected at Silchar, Cachar, Assam, among which I found four specimens of *M. fletcheri*. I have, therefore, been able to study this interesting species in more detail.

Musca senior-whitei. This species, as noted in my earlier paper (1932), might be placed either at the end of Group II or at the head of Group III. The posterior part of the paramere is not developed into a long bent process, the posterior process of the phallosome is, however, broadly expanded and slightly forked. Unfortunately, I have only been able to examine the terminalia of one male, as most of the type material consists of females. The proboscis (fig. 9) also exhibits an interesting stage in the evolution of the blood-drawing apparatus. The mentum is strongly chitinised, and has a well-developed posterior ridge; the keel is quite well developed and is rather long. The dorsal arms of the prestomal sclerite (fig. 10, *d*, *e*) are long and pointed, the remaining portion being very short, and the axial apophysis is not developed into a process, but is seen as a rounded dark area in the middle line; there is no dental plate, the part to which the teeth are attached

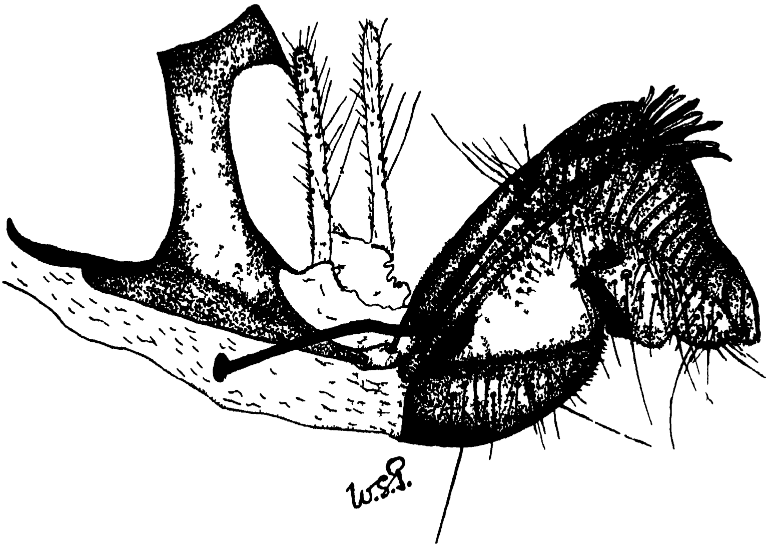

FIG 9 Proboscis of *M senior-whitei* with prestomal sclerite in position when drawing blood, note everted teeth and blades

being convex distally. There are five long, narrow teeth closely attached to the distal edge of the prestomal sclerite, each by two processes; in the position of action (fig. 9) they project freely from the membrane and are then seen as narrow blades surrounding the prestomum. The interdental armature consists of a pair of blade-like processes between each tooth, connected to a long rod attached between the teeth; they are longer than the teeth. (figs. 9, 10, *d*, *e*). This scratching apparatus is seen in action in fig. 9. It will be noted that the long, dorsal arms of the prestomal sclerite are drawn back, almost in a line with the labial gutter, forming a very acute angle with it (cf. fig. 9 with fig. 5, *a*, *b*); when the pseudotracheal membrane is drawn back, the teeth and blades are erected and everted through a wide arc; they scratch and tear the skin, and eventually draw blood. Although I have not seen this fly feeding, I have no doubt, from the structure of its proboscis, that it is a blood-sucker. I hope that some observer in India who has an opportunity of studying its habits in the field will settle this point; the type material was collected from cattle at Bezwada. The proboscis of *M. senior-whitei* exhibits a curious stage of development in the evolution of the blood-drawing apparatus. The free arms of the prestomal sclerite are long, the remaining part short, the axial apophysis undeveloped, and the teeth attached directly to the convex margin of the sclerite. But the general shape of the sclerite, the structure of the teeth and that of the interdental armature, in particular, suggest affinities with the other species of this group, rather than with *conducens* and *crassirostris*.

Musca inferior. The proboscis of this species is illustrated in fig. 11. The mentum is longer, as strongly chitinised, but not so deep (bulbous) as is that of *crassirostris*. It has a strong ridge at the posterior end for muscle attachment. The keel on the labial gutter is deep and projects into the mentum. The prestomal sclerite (fig. 10, *a*, *b*) is relatively short and wide, the dorsal free arms wide and bluntly pointed, while the end is strongly arched and carries the teeth and interdental armature. The axial apophysis is a long, stout, pointed process which projects well in front of the teeth in the position of action (fig. 5, *a*). There are usually five teeth, attached in a semi-circle to the dental plate, and one is usually smaller than the others (fig. 10, *a*); there is sometimes also a smaller sixth (fig. 10, *b*). The teeth are heavily chitinised blades, their apices pointed and strongly serrated (fig. 10, *c*). The interdental armature consists of two to three—mostly three—strong, knife-like or leaf-like blades arising from a common stalk between the teeth; they are always associated with a pseudotrachea, which is much reduced in calibre and does not pass down between the teeth. These blades project beyond the teeth. On comparing the prestomal

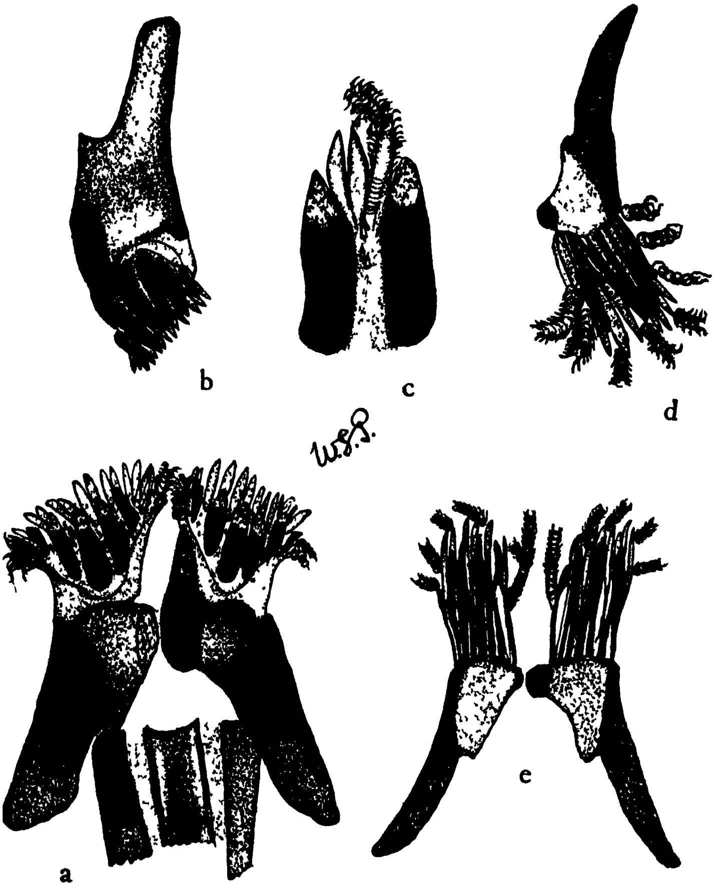

FIG. 10. *a.*—Prestomal sclerite of *M. inferior*, split and spread out to show inner surfaces of two halves; note end of labial gutter, rods, axial apophysis, teeth and blades of interdental armature; *b.*—prestomal sclerite of same in side view to show teeth, etc., of one half; *c.*—median teeth, three blades and one pseudotrachea of same enlarged; *d.*—prestomal sclerite of *M. senior-whitei* in side view to show teeth, etc., of one half (drawn to same scale as *b*); *e.*—prestomal sclerite of *M. senior-whitei*, split and spread out to show inner surfaces of two halves; note absence of dental plate and small size of axial apophysis.

sclerite of *M. fletcheri* and *M. inferior*, it will be noted that they closely resemble each other, both possessing a strongly developed, tooth-like axial apophysis; yet in *inferior* the interdental armature is well developed, whereas in *fletcheri* it is not. In *M. senior-whitei*, the interdental armature is strongly developed, consisting of very similar, though longer, blades than those of *inferior*. The rod-like structures of the interdental armature of *M. mesopotamiensis* suggest an early stage in the development of these blades. It is important to note that there is no indication of the formation of the serrated blades, so characteristic of the interdental armature of *crassirostris* and their early development in *conducens*, in any of these four species.

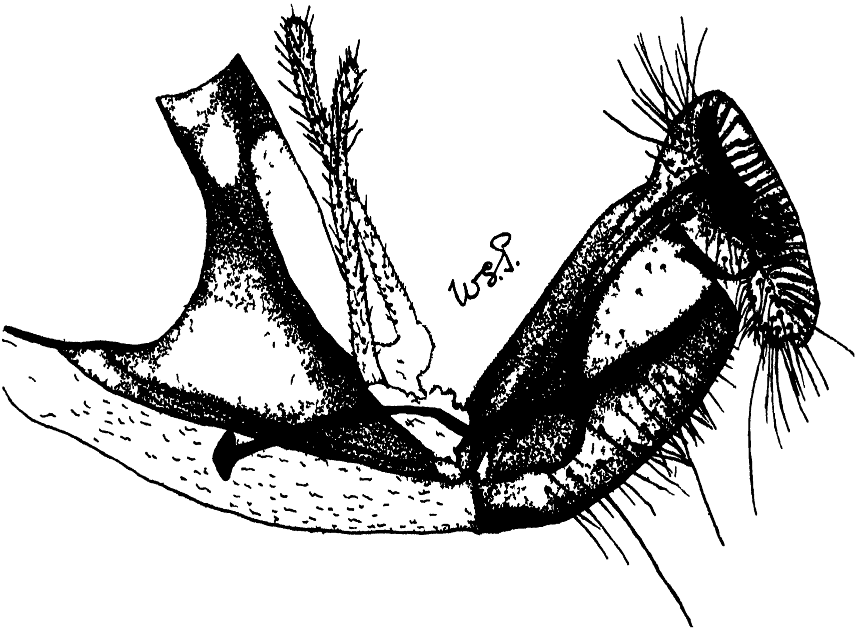

Fig. 11. Proboscis of *M. inferior*; note the large mentum, deep strong keel and labella folded back in position of rest.

The mechanism of the proboscis of *M. inferior* (fig. 5, *a*) when drawing blood is much the same as in *M. crassirostris* (fig. 5, *b*). The dorsal arms of the prestomal sclerite are drawn back, so that they come to lie almost in a line with the labial gutter. The pseudotracheal membrane is then retracted, the blades and teeth are everted and drawn across the skin, which is rapidly torn, and blood is drawn ; the axial apophysis projects forwards, its point fixing the prestomal sclerite.

In my next paper in this series, I hope to examine the status of the many genera erected in *Stomoxys* (sens. lat.), and to revise the species with the aid of the characters of the male terminalia which have proved to be of such value in *Musca*. Further, there is at the present time no satisfactory guide to the species of this important group of the blood-sucking Muscini.

I have also in preparation a revision of the genera of the subfamily Calliphorinae, based on a comparative study of the male terminalia. This will be followed by a revision of the species of the genera, especially *Lucilia*, *Calliphora* and *Chrysomyia*, many of the early stages of which have been collected.

I would like to take this opportunity of thanking Dr. R. Green and Mr. E. P. Hodgkin for large collections of *Stomoxys*, *Musca*, etc., from Kuala Lumpur ; Mr. G. B. Purvis, F.R.C.V.S., for collections from Alor Star, Malaya ; Mr. M. O. T. Iyengar, for species of *Stomoxys* and *Musca* from Travancore ; Mr. A. Cuthbertson, Mr. Munro, Dr. L. de Wulf, Mr. Symes, Mr. Hopkins, Mr. Hancock and Mr. Gibbins, for collections from various parts of Africa. I would again appeal to Medical Officers, entomologists and others for specimens of some of the rarer species of *Stomoxys*, especially those found on game animals. The specimens may be sent either preserved in spirit, or dried and then placed in soft paper, no wool or preservative, such as naphthaline, should be used in the packing. A list of species, of which more specimens are required, will be found in my previous paper, a copy of which will be sent to anyone desiring it. The following species were not included in the list :—

Species	Locality where found
Stomoxys spinigera Malloch ...	... Budda, Uganda ; Ruo, Nyasaland.
Stomoxys angustifrons Malloch	... Ngxwala Hill, Zululand.
Stomoxys hirtifrons Malloch ...	... Near Rumurti, Kenya Colony.
Stomoxys latifrons Malloch ...	... Ruo, Chiromo, Nyasaland (biting cattle) ; Ntambanana, Zululand.
Stomoxys punctigera Austen ...	... Uganda ; Belgian Congo. The male is unknown.
Stomoxys austeni Malloch ...	... Near Sandakan, Samawang, N. Borneo (in jungle).
Stomoxys transvittata Villeneuve	... Durban, S. Africa.

CONCLUSIONS

1. The generic characters of *Musca* are re-defined, and the significance of the structure of the male terminalia, as a guide to those of the higher forms, is briefly noted.

2. The proboscis of each of the seven species exhibiting various changes in the development of the blood-drawing apparatus is illustrated, and the changes in the parts concerned with scratching and tearing the skin of the host are briefly noted. A comparison of these with those of *M. domestica* demonstrates the fact that the differences in the prestomal sclerite, teeth, interdental armature and the structures associated with their function are those of degree and not of kind. The proboscis of each is in all other respects identical with that of *domestica*.

3. The blood-drawing apparatus in *Musca* is essentially of a scratching and tearing nature, the ends of the interdental armature and teeth, which vary structurally according to the group to which the flies belong, only enter the skin. It is, therefore, strictly not a piercing apparatus.

4. This study of the proboscis confirms the conclusions already reached as to the relationships of these species. But conversely it should be noted that the study of the proboscis by itself might quite easily lead to erroneous conclusions as to their affinities.

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STUDIES IN CHEMOTHERAPY*

X. FURTHER OBSERVATIONS ON THE TRANSMISSIBILITY OF TRYPARSAMIDE-RESISTANCE BY GLOSSINA

BY
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FREDERICK MURGATROYD
AND
FRANK HAWKING

(*Received for publication* 13 *March*, 1933)

In a previous communication (1933) we have referred in detail to the work of several investigators—Gonder (1911), Duke (1927) and Reichenow and Regendanz (1927)—who have endeavoured to determine whether the acquired character of arsenic-resistance in trypanosomes is transmitted through the intermediate invertebrate host. We have shown that the different conclusions reached by these workers were all unconvincing, because there is reason to believe that their so-called resistant strains were in fact but little, if any, more resistant than were the normal parent strains from which they were derived. We then gave a brief account of experiments performed by us, which, in our opinion, afforded conclusive proof that a strain of trypanosomes made highly resistant to tryparsamide preserved that resistance unchanged after cyclical transmission by *Glossina morsitans*. In the present paper we propose to describe these experiments in detail, and to record later observations on the same subject.

SOURCE AND CHARACTERS OF TRYPANOSOME STRAINS

Normal strain :—The trypanosome used in these experiments was a strain of *T. brucei* obtained by Dr. A. R. D. Adams in South Ankole, Uganda, by exposing a dog to the bite of wild *Glossina morsitans* in April, 1931. This strain has since been maintained by passage through guinea-pigs, as is shown in Table I. At the

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* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

TABLE I

Showing the history of the parent strain of *T. brucei* and of its tryparsamide-resistant branch, and the passage of each through Glossina.

Fly-transmitted normal strain		Parent strain		Fly-transmitted resistant strain			
		Main stem	Tryparsamide-resistant branch		First passage		Second passage
		Wild *Glossina morsitans*					
		Dog Passage 1 (April, 1931)					
		Guinea-pig 870 " 2					
		" 121A " 3					
		" 121B " 4					
		" 1A " 5					Guinea-pig 146B†
Guinea-pig 820C		" 2A " 6					" 224A
" 887A		" 3B† " 7				*G. palpalis* >	" 279A
" 887B	< *G. palpalis*	" 103B " 8					" 279B
" 942A		" 103C " 9			Guinea-pig 14B†		Mice
" 942B		" 681B " 10			" 14C	→	
		" 681C " 11 →	Guinea-pig 681C*†		" 67A		Guinea-pig 146B†
Guinea-pig 820A	<	← 681E† " 12 (Sept., 1932)	" 946	→ *G. morsitans* →	" 62A		" 224A
" 821A†		" 795D† " 13	" 966†		" 67B	*G. morsitans* >	" 251A
" 821B†		" 98A† " 14	" 74B†		Mice		" 251B
" 821C†		" 98D† " 15					" 251C
" 886A	< *G. morsitans*						Mice
" 886B							
" 919A							
" 919B†							
Mice							

* Infection made resistant by repeated doses of tryparsamide (Table III).

† From these guinea-pigs mice were inoculated to test the sensitiveness of the various strains to arsenicals.

time the experiment commenced (September, 1932), the parasite was a typically polymorphic trypanosome, and exhibited numerous posterior nuclear forms. Its virulence for guinea-pigs, rats and mice was similar to that of other recently isolated strains of *T. brucei* or *T. rhodesiense*: it killed guinea-pigs in eight to twelve weeks, and mice in four to eight weeks. Trypanosomes were usually to be found in considerable numbers in the peripheral blood of these animals throughout the course of the disease. In fact the strain was indistinguishable from other strains of *T. brucei* recently isolated from Glossina, and there is no reason to believe that in these respects (morphology and pathogenicity) the parasite had been in any way modified by its passage in the laboratory through a series of 11 or 12 guinea-pigs during a period of about 18 months.

With the object of ascertaining the response of the normal parent strain to various arsenical compounds, a number of mice and guinea-pigs were subinoculated from animals of the main-passage series—viz., from Guinea-pig 3B, 681E, 795D, 98A or 98D (Table I).

The results obtained in guinea-pigs were as follows:—Three guinea-pigs were treated with 0·05 gm. of tryparsamide per kilo. of body weight. In two of them parasites disappeared from the blood, which remained negative for 3 and 12 days respectively; in the third, which at the time of treatment showed a heavy infection, the blood did not become negative; in other animals smaller doses failed to clear the peripheral blood. It was thus concluded that the minimum effective dose of tryparsamide for guinea-pigs was approximately 0·05 gm. per kilo.

Details of the results of treating infected mice with tryparsamide, reduced tryparsamide, arsacetin and halarsol are given in Table II. It is of importance to note that all the mice referred to in this table were infected from guinea-pigs of the normal parent strain (*vide* Table I) and not from one another. Recent observations have suggested to us that the manner in which a strain has been previously passaged in laboratory animals may exert a considerable influence on the results obtained in experiments, the object of which is to determine the response of that strain to therapeutic substances. It must also be borne in mind that the present strain differed markedly from most of the laboratory strains which have been used for therapeutic experiments, in so far as its virulence in mice was

TABLE II

Showing the response to arsenicals in mice of the normal parent strain of *T. brucei*.

Drug	Dose per 20 gm. mouse mgms.	Source of infection (*vide* Table I)	Degree of infection of mice when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Tryparsamide	6·0	Guinea-pig 795D	10 to 20	4	0	0	1	1	1 mouse : 2
	8·0	Guinea-pig 98A	3 to 5	5	4	4	4	4	3 mice : 5 1 mouse : 9
	10·0	Guinea-pig 795D	1 to 20	7	2	6	7	...	1 mouse : 2 4 mice : 6 1 mouse : 13 1 mouse : 17
	20·0	Guinea-pig 795D	1/5 to 20	4	4	...	...	...	1 mouse : 7 2 mice : 10 1 mouse : 24
	30·0	Guinea-pig 795D	1 to 20	4	4	...	...	...	1 mouse : 11 1 mouse : 13 1 mouse : 17 1 mouse : cured
	40·0	Guinea-pig 795D Guinea-pig 98D	10 to 20	9	9	...	...	...	1 mouse : 2† 1 mouse : 8† 1 mouse : 13† 2 mice : 20† 1 mouse : 30 3 mice : cured
Reduced Tryparsamide	0·04	Guinea-pig 795D	2 to 10	5	0	1	1	1	1 mouse : 5
	0·06	Guinea-pig 795D	2 to 10	6	2	3	4	4	2 mice : 3 1 mouse : 4 1 mouse : 5
	0·08	Guinea-pig 98A	1 to 5	5	0	2	2	2	1 mouse : 1 1 mouse : 2
	0·1	Guinea-pig 3B Guinea-pig 795D	2 to 5	7	3	4	6	6	2 mice : 2 2 mice : 6 2 mice : 8
	0·125	Guinea-pig 795D	1 to 3	5	5	...	...	...	1 mouse : 7 2 mice : 9 2 mice : 12
	0·25	Guinea-pig 3B Guinea-pig 795D	1/5 to 20	8	8	...	...	...	1 mouse : 2† 1 mouse : 5 3 mice : 9 1 mouse : 12 1 mouse : 20 1 mouse : 22
	0·5	Guinea-pig 795D	1 to 10	5	5	...	...	...	1 mouse : 10 1 mouse : 11 1 mouse : 12 2 mice : 14

† Signifies mouse died without relapse.

TABLE II—*Continued*

Drug	Dose per 20 gm. mouse mgms.	Source of infection (*vide* Table I)	Degree of infection of mice when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Reduced Tryparsamide	1·0	Guinea-pig 795D	2 to 10	6	6	...	...	...	1 mouse : 3† 2 mice : 9 2 mice : 14 1 mouse : 17†
	1·5	Guinea-pig 98D	1/2 to 15	5	5	...	...	...	2 mice : 8 1 mouse : 10 1 mouse : 23 1 mouse : 30
	2·0	Guinea-pig 795D	1/10 to 10	5	5	...	...	...	1 mouse : 1† 1 mouse : 4† 1 mouse : 11† 1 mouse : 17 1 mouse : 19†
Arsacetin	3·0	Guinea-pig 98D Guinea-pig 795D	5 to 10	7	0	1	6	6	3 mice : 1 3 mice : 5 1 mouse : 7
	4·0	Guinea-pig 98A Guinea-pig 98D	1 to 20	7	0	2	7	.	4 mice : 1 2 mice : 5 1 mouse : 7
	5·0	Guinea-pig 795D	2 to 10	5	0	4	5	...	1 mouse : 2 3 mice : 4 1 mouse : 5
	6·0	Guinea-pig 795D	1 to 20	4	1	3	3	3	1 mouse : 3 1 mouse : 5 1 mouse : 6
	10·0	Guinea-pig 98D	+ to ++	5	5	...	...	...	4 mice : 8 1 mouse : 11
	20·0	Guinea-pig 98D	5 to ++	5	5	...	...	...	1 mouse : 6 3 mice : 8 1 mouse : cured
Halarsol	0·03	Guinea-pig 98A	1 to 10	5	3	5	...	...	2 mice : 2 2 mice : 4 1 mouse : 5†
	0·4	Guinea-pig 98D	20 to +	5	5	...	...	...	1 mouse : 6 3 mice : 8 1 mouse : 8†
	0·6	Guinea-pig 98D	20 to +	5	5	...	...	...	2 mice : 8 1 mouse : 9 1 mouse : 12 1 mouse : 14

† Signifies mouse died without relapse.

relatively mild; the average duration of the infection in these animals was from 1 to 2 months, and, although trypanosomes were usually to be found in the blood in large numbers, in an occasional mouse they disappeared spontaneously from the peripheral circulation for a day or two. These facts of necessity made the determination of the minimum effective dose of a drug more difficult in the case of the present strain than in that of acutely pathogenic strains such as our old laboratory strain of *T. rhodesiense.* With this difficulty in mind, we have indicated in the Table the number of animals which had become negative within 24, 48, 72 and 96 hours respectively after the administration of the drug.

It will be seen from Table II that in the case of mice the M.E.D.* of tryparsamide was about 8 mgm.; of reduced tryparsamide 0·06 to 0·1 mgm.; of arsacetin 3 mgm.; and of halarsol 0·03 mgm. per 20 gm. mouse. No attempt was made to determine the curative dose of tryparsamide in guinea-pigs; and attempts to ascertain the minimum curative doses of the various drugs in mice were unsuccessful, because in each instance the maximum dose tolerated by mice failed to produce cures in the majority of animals. These results are in striking contrast with those obtained previously with our old laboratory strain of *T. rhodesiense.* Whilst the M.E.D. of each drug for the present strain is only slightly greater than that for *T. rhodesiense,* the minimum curative dose is immensely greater and in fact coincided with, or even exceeded, the minimum dose tolerated by the mouse. The explanation of this very interesting point is obscure and we shall return to it in a later paper.

Resistant variety :—With the object of preparing a strain resistant to arsenicals, Guinea-pig 681C (after the main strain had been passed on into Guinea-pig 681E) was treated with repeated small, but gradually increasing, doses of tryparsamide. Details are given in Table III, from which it will be seen that a stage was finally reached when a dose of 0·5 gram per kilo. failed to clear the blood of parasites. As this dose approached the maximum tolerated by a guinea-pig, and as it was ten times the dose which we had found to be the M.E.D. for the normal parent strain, it was decided at this point to stop the administration of tryparsamide, and to test the response of the strain to arsenicals in subinoculated guinea-pigs and mice.

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* Minimum Effective Dose (M.E.D.). The minimum dose which sufficed to clear the blood of at least 80 per cent. of infected mice, within a period of 4 days.

The results obtained in guinea-pigs were as follows:—Three animals were given doses of 0·5 gm. of tryparsamide per kilo. and in none of them did the blood become negative; four others were given 1·0 gm. per kilo., and in two of them the blood became negative for 1 and 5 days respectively; one of the four died in 5 days from the toxic action of the drug. It is thus clear that the infection in guinea-pigs resisted the maximum dose of tryparsamide well tolerated by these animals (i.e. 0·5 gm. per kilo.).

TABLE III

Showing the production of the tryparsamide-resistant branch

Guinea-pig 681c	September			October																
	21	22–29	30	1	3	4	5	6	7	8	10	11	12	13	14	15	17	18	20	21
Blood infection ...	+	−	$\frac{1}{8}$	3	3	$\frac{1}{20}$	$\frac{1}{10}$	1	5	10	20	10	10	5	2	10	20	−	$\frac{1}{10}$	5
Tryparsamide ...	0·125	...	...	0·04	0·04	...	...	0·04	0·04	0·06	0·08		0·1	. .	.	0·15	0·2	...	...	0·15

Guinea-pig 681c	October						November													
	22	24	25	26	27	28–31	1	2	3	4	5	7	8	9*	10	11	12	14	15	16
Blood infection ...	$\frac{1}{8}$	$\frac{1}{5}$	$\frac{1}{2}$	$\frac{1}{50}$	2	−	$\frac{1}{10}$	2	10	20	30	++	3	20	20	30	30	20	20	20
Tryparsamide ...	...	...	0·15		0·2	...		0·15	.	0·2	0·2	0·5	. .	..	...	. .	...	..	...	...

N.B.—Blood infection: the figures indicate the number of trypanosomes per microscope field.
Tryparsamide: the figures indicate dose in grams per kilo. of guinea-pig.

* On this date animals were subinoculated to test the resistance of the strain to arsenicals, and the feeding of Glossina commenced.

The results of treating infected mice with the various drugs are shown in Table IV, from which it will be seen that 45 mgm. of tryparsamide, 2 mgm. of reduced tryparsamide, 30 mgm. of arsacetin, and 0·6 mgm. of halarsol per 20 gm. mouse were ineffective. It should be noted that these doses represent approximately the maximum doses of the various drugs tolerated by the majority of mice. Comparison of these figures with those previously given for the normal parent strain shows that the resistant variety had thus attained to a very high degree of resistance. It is interesting to observe that this pronounced change in the resistance of the strain to aromatic arsenicals was not accompanied by any discernible alteration in its morphology or pathogenicity.

SOURCE OF GLOSSINA AND ITS MAINTENANCE IN THE LABORATORY

In June, 1932, we wrote to Dr. H. L. Duke of Uganda and to Dr. J. F. Corson of Tanganyika, asking them to be good enough to send by air mail regular supplies of pupae of *Glossina palpalis* and *Glossina morsitans* respectively. During the past six months we have received from Dr. Duke one consignment of 1,000, and seven, each of about 500, *G. palpalis* pupae collected on the shores of Lake Victoria ; and Mr. Jackson, at Dr. Corson's request, sent us one large consignment of about 3,000 pupae of *G. morsitans* collected at Kikori. To these gentlemen our thanks are due.

TABLE IV

Showing the response to arsenicals in mice of the tryparsamide-resistant branch of the parent strain.

Drug	Dose per 20 gm. mouse mgm.	Source of infection (*vide* Table I)	Degree of infection of mice when treated. Parasites per field 1/6 obj. 4 oc	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Tryparsamide ..	45·0	Guinea-pig 966 " 74B	3 to 20	7	0	1	1	1	1 mouse : 3
Reduced Tryparsamide ..	2·0	Guinea-pig 681c " 74B	1/3 to 20	5	0	0	0	0	—
Arsacetin	30·0	Guinea-pig 966	5 to 20	5	0	0	0	0	—
alaisol	0·6	Guinea-pig 966 " 74B	1 to +	5	0	1	1	1	1 mouse : 1

The first consignment, which consisted of 1,000 pupae of *G. palpalis*, was sent in a wooden container of such small size that the pupae almost completely filled it ; the container was wrapped in brown paper. When the package reached Liverpool, 9 days after its dispatch from Entebbe, a small number of dead flies was found amongst the pupae, thus indicating that a few pupae had hatched en route. The remainder of the pupae, which appeared to be perfectly normal, were placed in a large cage constructed of wood and wire gauze ; this was kept in a glass-house, the average daily maximum temperature of which was 86° F. and the average nightly minimum, 60° F. The pupae were exposed to strong daylight, but were protected from the direct rays of the sun. To our disappointment not a single fly emerged from this very large consignment of apparently healthy pupae.

In casting round for an explanation of this misfortune, it occurred to us that a reasonable solution was that the pupae had all died during the journey from Africa from want of oxygen. We accordingly wrote again to Dr. Duke and asked him to send future consignments in ordinary cigarette tins, loosely packed with cotton wool to prevent mechanical damage, and to arrange that a number of small perforations were made through the tin and paper wrapping, so as to ensure a plentiful supply of air. All further consignments were packed in this way, and all survived the journey well, as from each large numbers of flies were obtained.

In our earlier experiments, which were conducted during the summer and early autumn, the tsetse flies were hatched and kept in the glass-house referred to above, but as the season advanced it became increasingly difficult to maintain the glass-house at a temperature sufficiently high for transmission experiments. Consequently we were compelled to transfer the flies to an incubator kept at 80° F. to 84° F. during the nights ; and finally we had to abandon the glass-house altogether and to rely entirely on incubators.

The exclusive use of incubators for the hatching and maintenance of Glossina presents certain difficulties which are not met with when the glass-house is used. Roubaud (1917) many years ago drew attention to this fact. He stated that for the normal development of the tsetse it was essential to renew the air in the incubator ; in closed incubators the flies did not develop their wings on emerging from the pupae, and furthermore many of the pupae did not hatch. Roubaud adds that he surmounted this difficulty by leaving the incubator slightly open. One of us and Macfie (1924), working with

G. tachinoides, the pupae of which had been sent to Liverpool, likewise observed that the flies which had hatched in the incubator failed to expand their wings and remained incompletely developed. These observers believed this lack of proper development to be due to absence of light. Present experience has confirmed this view that light is essential for the complete development of freshly-hatched tsetse. Insects which emerge, and are subsequently kept in dark incubators, do not expand their wings and are unable to feed.

Accordingly, in the winter months, when low temperatures prevented the use of the glass-house, we found it desirable to use two incubators. In the one in which the flies were kept during the night (5 p.m. to 10 a.m.) both glass and wooden doors were closed and the incubator kept at a temperature of from 82° to 84° F. In the day time (10 a.m. to 5 p.m.) the flies were transferred to a second incubator the glass door of which was closed, but the wooden door was left open; the glass door was directed towards a southern window, and the interior of the incubator was lined with white paper in order to ensure that the flies were exposed to as much light as possible; the temperature of this incubator was kept at 90° to 95° F. These high temperatures are unfavourable to the fly, but they facilitate the development of the trypanosome in the insect. This fact was shown many years ago by Kinghorn and Yorke (1912); and it has recently been stated by Taylor (1932) that the exposure of *G. tachinoides* to very high temperatures, i.e., approaching 98° F. for a limited period each day, materially increased the percentage which become infective.

It is, of course, a matter of the utmost importance that the humidity of the atmosphere should be kept as high as possible, otherwise the flies will die rapidly when kept at temperatures of over 80° F. In the glass-house we had a mechanical device for sprinkling water over the hot pipes, and in the incubator the humidity of the atmosphere was maintained by the use of large flat dishes containing water.

Furthermore, it is important to use scrupulously clean incubators. On one occasion we lost about 40 flies owing to the fact that the incubator had previously contained a tube with tissues clearing in clove oil preparatory to being embedded. Although this had been removed some time before the incubator was used for the tsetse-fly work, a faint smell of the clove oil was still noticeable, and within 12 hours about 90 per cent. of the flies had died. Further experience showed that slight traces of a volatile substance, such as clove oil, cedar oil or formalin, were highly injurious to Glossina.

Provided these precautions were taken, we found it possible to keep both *G. palpalis* and *G. morsitans* alive in incubators at temperatures varying from 80° to 95° F. for a sufficient length of time to conduct successful transmission experiments.

TRANSMISSION EXPERIMENTS

In the transmission experiments, the flies were fed 3 or 4 times on infected guinea-pigs at 3 or 4 day intervals, and subsequently on a series of healthy guinea-pigs. In the present paper we are concerned with three experiments, in each of which both *G. palpalis* and *G. morsitans* were employed under identical conditions.

In Experiment I an attempt was made to transmit the normal parent strain of *T. brucei*. In all, 91 *G. palpalis* and 90 *G. morsitans* were fed on 4 occasions on Guinea-pig 681E (Table I), and subsequently each species of tsetse was fed on a series of normal guinea-pigs.

In Experiment II an attempt was made to transmit the branch of the parent strain made resistant to tryparsamide, and 58 *G. palpalis* and 51 *G. morsitans* were fed on 4 occasions on Guinea-pig 681C or 946 (Table I). Each species of fly was then fed on a series of normal guinea-pigs.

In Experiment III an attempt was made to transmit the tryparsamide-resistant branch which had already been passed once through Glossina in Experiment II. In this experiment 160 *G. palpalis* and 6 *G. morsitans* were fed on 4 occasions on Guinea-pig 14B, 14C or 67A (Table I); the flies were then fed on a series of normal guinea-pigs.

The results of these transmission experiments are summarized in Table V.

Table V shows the number of flies at the commencement of the experiment, i.e., at the time of the first infected feed, and also the number alive at the time of the first feed on a clean animal. It will be seen that in each experiment a considerable number of

TABLE V

Summarizing the results of the transmission experiments.

Strain	Species of Glossina	No. of Glossina		Result of dissections.* No. positive		Results of feeding flies on series of healthy animals: − not infected + infected
		At beginning of experiment	At time of first feed on clean animal	Gut only	Gut and salivary glands	
Parent ... ... (Guinea-pig 681E)	*palpalis*	91	71	3	1	Guinea-pig 820B −
						„ 820C +
						„ 887A +
						„ 887B +
						„ 942A +
						„ 942B +
	morsitans	90	59	8	2	Guinea-pig 820A +
						„ 821A +
						„ 821B +
						„ 821C +
						„ 886A +
						„ 886B +
						„ 919A +
						„ 919B +
						†mice +
Tryparsamide-resistant-branch 1st passage (Guinea-pig 681C) (Guinea-pig 946)	*palpalis*	58	26	0	0	Guinea-pig 14A −
						„ 62B −
	morsitans	51	24	1	1	Guinea-pig 14A −
						„ 14B +
						„ 14C +
						„ 62A +
						† „ 67A +
						† „ 67B +
						† mice +
Tryparsamide-resistant-branch 2nd passage (Guinea-pig. 14B) (Guinea-pig. 14C) (Guinea-pig 67A)	*palpalis*	160	108	6	2	Guinea-pig 146A −
						„ 146B +
						„ 224A +
						† „ 279A +
						† „ 279B +
						mice +
	morsitans	6	4	0	1	Guinea-pig 146A −
						„ 146B +
						„ 224A +
						† „ 251A +
						† „ 251B +
						† „ 251C +
						† mice +

* This relates only to flies dissected after the first feed on a clean animal.

† In these animals infection was produced by the inoculation of metacyclic trypanosomes from the salivary glands of infective flies.

flies died during this interval, which was, as a rule, 12 to 16 days. No account is taken of flies which died before the first clean feed, and the figures relating to positive flies refer only to those dissected after the first clean feed. In the last column of the Table a list is given of the clean animals on which each species of tsetse was fed.

It will be seen that each experiment was successful, in that the trypanosome completed its biological development in at least one fly, and a series of clean animals was infected by the bite of the infected insect. Under the conditions of experiment—the flies were fed on the infected animals on several occasions over a considerable period—it is obvious that it is impossible to determine the exact period occupied by the cycle of the trypanosome in Glossina, i.e., the period between the time of the actual infecting feed and that at which the insect was first capable of infecting clean animals; analysis of the data available, however, indicates that in two flies it could not have been more than 14 days, and that in others it was between 14 and 24 days.

In each experiment the results obtained with *G. palpalis* can be compared with those obtained with *G. morsitans*, as with both species the conditions of experiment were identical. The total number of flies is small, but the figures suggest that *G. morsitans* is more susceptible to infection by the strain of *T. brucei* used than is *G. palpalis*: thus, of 205 *G. palpalis* which were alive at the time of the first feed on a clean animal, 9 (4·5 per cent.) showed an infection of the gut alone, and 3 (1·5 per cent.) showed an infection of both gut and salivary glands; and of 87 *G. morsitans*, 9 (10·4 per cent.) showed an infection of the gut alone, and 4 (4·6 per cent.) showed an infection of both gut and salivary glands. These figures are in agreement with the results published by Duke* elsewhere in this issue.

As will be seen from the Table, .we succeeded in our endeavours to transmit by Glossina each of the following three strains:—

(i) The normal parent strain.

(ii) The tryparsamide-resistant branch of the parent strain (1st fly passage).

(iii) The tryparsamide-resistant branch which had already been once transmitted by Glossina (2nd fly passage).

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* *Vide* p. 123.

RESPONSE OF THE TRANSMITTED STRAINS TO ARSENICALS

The response to arsenicals of the various transmitted strains was tested firstly in guinea-pigs directly infected by the bite of infective tsetse; and secondly in mice, either inoculated from these guinea-pigs or injected with metacyclic trypanosomes from the salivary glands of the infective tsetse.

The results obtained in guinea-pigs were as follows:—

(i) *Guinea-pigs infected with the normal strain passed through Glossina*:—Five guinea-pigs were given 0·05 gm. tryparsamide per kilo. In three of them the blood became negative for 2, 4 and 8 days respectively; and in two the infection was definitely reduced, but the blood did not become negative.

(ii) *Guinea-pigs infected with the tryparsamide-resistant strain passed once through Glossina*:—Three guinea-pigs were given 0·5 gm. tryparsamide per kilo. and one received 1·0 gm. per kilo. In none of these animals did the blood become negative.

(iii) *Guinea-pigs infected with the tryparsamide-resistant strain passed twice through Glossina*:—Three guinea-pigs were given 0·5 gm. tryparsamide per kilo. In two the infection was uninfluenced and in one the blood became negative for a single day only.

The results obtained in mice are set forth in Tables VI, VII and VIII. It is to be noticed that all the mice referred to in these Tables were infected either directly with the metacyclic trypanosomes from the salivary glands of the infective flies, or from the guinea-pigs infected by the bite of these flies. In this respect the Tables are as nearly as possible comparable with Tables II and IV. As already stated, we believe this point to be of considerable importance in therapeutic experiments, and we intend to discuss it fully in a later paper.

If Table VI be compared with Table II it will be seen that the results obtained with the normal strain after passage through Glossina are indistinguishable from those obtained with the same strain before its passage through Glossina; similarly if Tables VII and VIII be compared with one another and with Table IV it will be seen that two successive passages through Glossina had not produced

TABLE VI

Showing the response to arsenicals in mice of the normal parent strain after passage through Glossina.

Drug	Dose per 20 gm. mouse mgms.	Source of infection (*vide* Table I)	Degree of infection when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Tryparsamide	6·0	Guinea-pig 821C Guinea-pig 919B	2 to 20	9	1	4	6	7	1 mouse : 1 1 mouse : 2 1 mouse : 3 2 mice : 4 1 mouse : 5 1 mouse : 6
	8·0	Guinea-pig 919B	2 to 10	10	2	5	6	6	1 mouse : 4 2 mice : 5 1 mouse : 7 2 mice : 8
	10·0	Guinea-pig 821C Guinea-pig 919B	2 to 20	10	3	8	10	...	1 mouse : 4 4 mice : 5 5 mice : 6
	20·0	Guinea-pig 821C	1/5 to 3	5	5	...	...	...	1 mouse : 9 3 mice : 11 1 mouse : 15
	30·0	Guinea-pig 821C Guinea-pig 919B	1/3 to 10	11	11	...	...	...	1 mouse : 2† 1 mouse : 9 2 mice : 14 3 mice : 18 2 mice : 25 2 mice : cured
	40·0	Guinea-pig 821C Guinea-pig 919B	1/2 to 20	10	10	...	..	...	1 mouse : 3† 1 mouse : 8 1 mouse : 13† 1 mouse : 23 6 mice : cured
Reduced Tryparsamide	0·06	Guinea-pig 821A	1 to 10	5	0	2	2	2	1 mouse : 2 1 mouse : 5
		G. morsitans	1 to 20	4	1	2	2	2	1 mouse : 1 1 mouse : 2
	0·08	Guinea-pig 919B	1 to 15	10	7	10	...	...	1 mouse : 3 4 mice : 4 1 mouse : 5 1 mouse : 5† 1 mouse : 6 1 mouse : 7 1 mouse : 9
	0·1	Guinea-pig 821C	1 to 5	5	3	5	...	...	3 mice : 5 1 mouse : 6 1 mouse : 7
		G. morsitans	1 to 5	3	1	2	2	2	1 mouse : 2 1 mouse : 3
	0·125	Guinea-pig 821A Guinea-pig 821B	1 to 20	5	5	...	...	...	1 mouse : 5 1 mouse : 8 1 mouse : 10 2 mice : 11

† Signifies mouse died without relapse.

TABLE VI—*Continued*

Drug	Dose per 20 gm. mouse	Source of infection (*vide* Table I)	Degree of infection when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative
	mgms.				24	48	72	96	days
Reduced Tryparsamide	0·25	Guinea-pig 821A Guinea-pig 821B	1 to 10	5	3	5	...	...	1 mouse : 4 1 mouse : 7 3 mice : 14
	0·5	Guinea-pig 821A Guinea-pig 821B	1 to 10	5	4	5	...	...	1 mouse : 6 1 mouse : 8 1 mouse : 11† 1 mouse : 12 1 mouse : 12†
	1·0	Guinea-pig 821A Guinea-pig 821C	1/5 to 1	5	5	...	...	...	1 mouse : 6† 1 mouse : 9 1 mouse : 14 1 mouse : 16† 1 mouse : 22
		G. morsitans	5 to 10	3	3	...	...	...	2 mice : 13 1 mouse : 13†
	1·5	Guinea-pig 919B	1 to 3	5	5	...	...	...	1 mouse : 11 4 mice : cured
	2·0	Guinea-pig 821C	1/2 to ++	8	8	...	...	...	3 mice : 3† 1 mouse : 5† 2 mice : 10† 1 mouse : 13 1 mouse : 14†
Arsacetin	3·0	Guinea-pig 919B	5	5	1	1	1	2	1 mouse : 1 1 mouse : 19
	4·0	Guinea-pig 919B	10	5	1	4	4	4	1 mouse : 2 2 mice : 7 1 mouse : 8
	5·0	Guinea-pig 821C	2 to 20	5	0	5	...	...	2 mice : 4 1 mouse : 5 1 mouse : 6 1 mouse : 9
	6·0	Guinea-pig 821C	20 to +	2	2	...	...	...	1 mouse : 8 1 mouse : 10
	10·0	Guinea-pig 919B	1/2 to 10	5	5	...	...	...	3 mice : 6 1 mouse : 9 1 mouse : 13
	20·0	Guinea-pig 919B	1/3 to +	6	6	...	...	...	2 mice : 13 2 mice : 16 1 mouse : 25 1 mouse : cured
	30·0	Guinea-pig 919B	1/2 to 10	5	5	...	...	...	1 mouse : 1† 2 mice : 2† 2 mice : cured

† Signifies mouse died without relapse.

TABLE VI—*Continued*

Drug	Dose per 20 gm. mouse mgms.	Source of infection (*vide* Table I)	Degree of infection of mice when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Halarsol	0·02	Guinea-pig 919B	1 to 10	10	1	2	2	4	3 mice : 1 1 mouse : 2
	0·03	Guinea-pig 919B	1 to 10	13	8	12	12	12	1 mouse : 1 2 mice : 2 5 mice : 3 3 mice : 5 1 mouse : 6
	0·04	Guinea-pig 919B ...	1 to 20	10	8	10	...	...	1 mouse : 1 1 mouse : 4 1 mouse : 5 5 mice : 6 2 mice : 7
	0·08	Guinea-pig 919B	1 to 5	10	10	...	..	...	1 mouse : 5† 2 mice : 7 4 mice : 8 1 mouse : 9 2 mice : 11
	0·1	Guinea-pig 919B	5 to 10	4	4	...	...	...	1 mouse : 7 2 mice : 8 1 mouse : 9
	0·12	Guinea-pig 919B	1 to 10	10	10	...	...	...	2 mice : 8 2 mice : 10 3 mice : 13 2 mice : 18 1 mouse : 19†
	0·2	Guinea-pig 919B	1/2 to 5	5	3	5	...	...	2 mice : 6 1 mouse : 8 2 mice : 10
	0·4	Guinea-pig 919B	1 to 5	6	6	..	...	...	1 mouse : 2† 1 mouse : 9 1 mouse : 11 1 mouse : 15 1 mouse : 19 1 mouse : cured
	0·6	Giunea-pig 919B	1 to 5	5	5	...	...	...	1 mouse : 11† 1 mouse : 16† 2 mice : 18† 1 mouse : cured

† Signifies mouse died without relapse.

TABLE VII

Showing the response to arsenicals in mice of the tryparsamide-resistant strain passed once through Glossina.

Drug	Dose per 20 gm. mouse mgm.	Source of infection (*vide* Table I)	Degree of infection of mice when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Tryparsamide ..	45·0	Guinea-pig 14B	2 to +	9	2	2	2	2	1 mouse : 1 1 mouse : 1†
Reduced Tryparsamide ..	2·0	*G. morsitans*	3 to 10	5	0	0	0	0	—
Arsacetin	30·0	Guinea-pig 14B *G. morsitans*	20 5 to 20	2 3	0 0	0 0	0 0	0 0	—
Halarsol	0·6	Guinea-pig 14B	1 to +	5	0	0	0	0	—

TABLE VIII

Showing the response to arsenicals in mice of the tryparsamide-resistant strain passed twice through Glossina.

Drug	Dose per 20 gm. mouse mgm.	Source of infection (*vide* Table I)	Degree of infection of mice when treated. Parasites per field 1/6 obj. 4 oc.	Number of mice treated	Number of mice which had become negative in hours				Length of time mice remained negative days
					24	48	72	96	
Tryparsamide .	45·0	Guinea-pig 146B *G. morsitans*	5 to 10 10 to 20	3 2	0 0	0 1	0 1	1 1	1 mouse : 1 1 mouse : 1†
Reduced Tryparsamide .	2·0	Guinea-pig 146B *G. palpalis*	10 to 20 10 to 20	3 2	0 0	0 0	0 0	0 0	— —
Arsacetin	30·0	Guinea-pig 146B *G. morsitans*	1 to 20 10 to ++	2 3	0 0	0 0	0 0	0 0	— —
Halarsol	0·6	*G. morsitans* *G. palpalis*	10 to ++ 10	4 1	0 0	0 0	1 0	1 0	1 mouse : 2 —

† Signifies mouse died without relapse.

any change in the resistance of the tryparsamide-fast strain. We have summarized the minimum effective doses (M.E.D.) of the various drugs for the normal and tryparsamide-resistant strains, before and after transmission by Glossina, in Table IX, from which it will be seen that the reactions of the two strains (normal and resistant) to arsenicals were in no way modified by passage through Glossina; that is, the fly-transmitted normal strain was just as sensitive to arsenicals as was its normal parent strain, and the fly-transmitted resistant strains were just as resistant as was their resistant parent strain.

TABLE IX

Showing the M.E.D. of various drugs for mice infected with the normal and tryparsamide-resistant strains respectively, before and after passage through Glossina.

Drug	Normal strain		Tryparsamide-resistant strain		
	Parent	After passage through Glossina	Parent	After one passage through Glossina	After two passages through Glossina
Tryparsamide...	8	6 10	45+	45+	45+
Reduced Tryparsamide ..	0·06–0·1	0·08	2+	2+	2+
Arsacetin	3	4	30+	30+	30+
Halarsol	0·03	0·03	0·6+	0·6+	0·6+

N.B.—The figures represent milligrams of drug per 20 gm. of mouse.

We have already referred to the fact that there is no reason to believe that at the time our experiments commenced the parent strain had undergone, as the result of its 18 months' sojourn in a series of 12 to 13 guinea-pigs, any change from its original condition in respect of morphology and pathogenicity. The strain exhibited the morphological characters, and virulence for mice and guinea-pigs, which we are accustomed to regard as typical of *T. brucei* or *T. rhodesiense* freshly isolated from Glossina. As might be expected, passage of this normal strain through Glossina produced no change in its apparently normal characters. It is, however, of greater interest to note that the production of the tryparsamide-resistant variety in Guinea-pig 681C (Table III) was not accompanied by any discernible change in morphology or pathogenicity, and that the transmissibility of the resistant strain by Glossina was apparently unimpaired; in this case also the strain was, so far as could be ascertained, exactly the same after passage through Glossina as before, i.e., it exhibited the same high degree of resistance to the aromatical arsenicals and the same normal characters of morphology and pathogenicity.

SUMMARY

1. In this paper a detailed account is given of experiments devised with the object of ascertaining whether a tryparsamide-fast strain of trypanosomes is transmissible by Glossina, and if so whether its resistant character persists after passage through the fly.

2. The trypanosome used was a strain of *T. brucei*, isolated from wild *G. morsitans* in Uganda in 1931, and subsequently maintained in the laboratory by passage through guinea-pigs for a period of 18 months. Careful examination of the strain showed that in so far as its morphology and pathogenicity were concerned it was indistinguishable from other strains of *T. brucei* recently isolated from Glossina: this implies that the strain had undergone no discoverable change during its 18 months' sojourn in guinea-pigs. The response of the strain to arsenicals was then carefully determined: in guinea-pigs the M.E.D. of tryparsamide was about 0·05 gm. per kilo; and in mice the M.E.D. of tryparsamide was 8 mgm., of reduced tryparsamide 0·06 to 0·1 mgm., of arsacetin 3 mgm., and of halarsol 0·03 mgm. per 20 gm.

3. The tryparsamide-resistant variety was made by repeated administration of gradually increasing doses of the drug to an infected guinea-pig; eventually the resistance of the strain was increased to a point when it withstood the maximum dose well-tolerated by a guinea-pig, i.e., about 10 times the M.E.D. for the normal strain. It is of importance to note that this change in drug-resistance was not accompanied by any change in morphology or pathogenicity. Experiments showed that in guinea-pigs this resistant strain withstood 0·5 gm. of tryparsamide per kilo; and in mice 45 mgm. of tryparsamide, 2 mgm. of reduced tryparsamide, 30 mgm. of arsacetin and 0·6 mgm. of halarsol per 20 gm. From this it follows that the strain resisted the maximum doses of the various drugs tolerated by the vertebrate hosts.

4. In the transmission experiments both *Glossina palpalis* and *Glossina morsitans* were used. The insects were sent to this country in the pupal state by air mail. An account is given of the methods employed for the maintenance of the flies in the laboratory, and of the technique adopted for the transmission experiments.

5. Transmission experiments were successful with :—

   (i) The normal parent strain.

   (ii) The tryparsamide-resistant branch of the parent strain. (1st fly-passage).

   (iii) The tryparsamide-resistant branch which had already been once transmitted by Glossina (2nd fly-passage).

Some evidence was obtained which suggests that the strain of *T. brucei* used was more easily transmissible by *G. morsitans* than by *G. palpalis*.

6. The transmitted strains were carefully examined as regards their morphology, pathogenicity and response to arsenicals. It was found that in none of these respects had the two strains been modified as the result of their passage through Glossina.

The normal strain after passage through Glossina was just as sensitive to arsenicals as it had been previously, and similarly two successive passages of the resistant-strain through Glossina had in no degree lessened its resistant characters.

7. These experiments afford, in our opinion, conclusive proof that :—

   (i) Strains of trypanosomes possessing a high degree of resistance to the aromatic arsenicals are transmissible by Glossina.

   (ii) The acquired character of drug-resistance is unimpaired after two successive passages through Glossina.

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MISCELLANEA

A NOTE ON THE VALUE OF ISSUING FREE QUININE TO NATIVE INFANTS

As shown in a recent note (Gordon and Davey, 1933), there has been a marked increase in the incidence of quartan malaria amongst children in Freetown. It has been the custom for Medical Officers in charge of infant clinics to supply with free quinine every infant brought to the clinic, regardless of whether it was suffering from malaria or not. The adult who brought the child was told to continue her attendance weekly, and was given seven powders of euquinine, each containing two grains, one powder to be administered to the child every day.

The results of examining the blood of the children under 1 year of age who had been supplied with quinine, and of those of similar ages attending the clinic for the first time and therefore not supplied with quinine, was as follows :—

Number of children, supplied with quinine a week or more previously, whose blood was examined	97
Percentage of these found positive	43
Number of children not supplied with quinine whose blood was examined	116
Percentage of these found positive	57

It was not found possible to examine the urines of these very young children, so that we have no knowledge as to whether the drug was regularly administered, but we are inclined to think it was not, since in our experience the uneducated native has a prejudice against the use of quinine. Our results therefore only show that this particular method of supplying quinine to the infant population in this Colony is an unsatisfactory one so far as prophylaxis is concerned, although we are informed that the incidence of grave infection has been reduced.

REFERENCE

GORDON, R. M., and DAVEY, T. H. (1933). A further note on the increase of *P. malariae* in Freetown, Sierra Leone. *Ann. Trop. Med. & Parasitol.*, **27**, 53.

R. M. GORDON.
T. H. DAVEY.

28 *November*, 1932.

IS TRYPANOSOMIASIS IN THE LACTATING RAT TRANSMITTED TO THE SUCKING YOUNG?

The object of the investigation was to answer this question. Four healthy female rats in various stages of lactation were inoculated with equal amounts of citrated blood infected with *T. brucei* (·25 c.c. of 1/100 blood), the blood being obtained from a mouse dying of the infection. The litters varied in age from 6 to 43 days. The two younger litters were limited to the mother's milk, while the two litters over 3 weeks old were starved in the evening and overnight, so that, when the mother was placed in their cages for a few hours in the morning, they invariably resorted to sucking.

All the lactating rats died from the infection in 8 to 9 days, the blood being positive in all cases 3 days after inoculation.

On the death of the mother rat with the youngest litter, a lactating fostermother was placed in a cage with them; the young of the other litters were put on a stock diet of fine ground oats made into a paste with milk and water.

Fate of the Young. The 3 young placed with the fostermother were eaten; one young from another litter died at the same time as its mother, but showed no evidence of trypanosomiasis either P.M. or when examined 3 days previously. All the others survived and grew normally, and were in good health when examined 14 days after the death of the last mother. None of them showed the presence of trypanosomes in the blood when examined a week after the blood of the mothers was found to be positive.

In one instance, milk was expressed from 6 of the mammary glands of a dying lactating mother, but no trypanosomes were found, although the blood was swarming with trypanosomes at the time. The findings are summarised in the following table.

Number of rat	Trypanosomes + in	Died in	Number of litter	Age of litter at beginning of experiment	Fate of young
23	3 days	8 days	5	43 days	Alive and well 14 days after death of mother.
47	3 days	9 days	3	6 days	Well, until eaten by foster-mother
64	3 days	8 days	1	17 days	Alive and well 14 days after death of mother.
65	3 days	9 days	5	25 days	4 alive and well 14 days after death of mother. 1 died at same time as mother—but no evidence of infection

CONCLUSION

Although the experiment was admittedly carried out on a limited scale, the results strongly indicate that trypanosomiasis in the female rat is not conveyed to the young in the process of lactation.

5 *October*, 1932. J. Fine, M.D., D.P.H., D.T.M.

TWO CASES OF ANIMAL RABIES OCCURRING IN SIERRA LEONE

Although rabies appears to be not uncommon in both Nigeria and the Gold Coast, we can find no previous record of its occurrence in the remaining British Colonies of Sierra Leone and the Gambia, in spite of the fact that the latter both lie adjacent to French Guinea, from which numerous cases have been reported.

Case I. In February, 1932, two Europeans in Freetown, together with their native servants, were bitten by a cat showing signs of rabies. Sections of the animal's brain showed the presence of Negri bodies in the ganglion cells.

Case II. In December, 1932, we examined the brain of a dog from Mano in the Southern Province, which had bitten four natives before it could be killed. Negri bodies were demonstrated in sections of the brain, and the disease transferred successfully to guinea-pigs. The infection of these guinea-pigs is of some interest since the virus survived a 36 hours' journey, without ice, in the hottest season of the year.

R. M. Gordon.
E. P. Hicks.
T. H. Davey.

7 February, 1933.

THE IDENTITY OF *CULEX AEGYPTI* L.

In the recent edition of the 'Genera Insectorum' (1932), Dr. Edwards has changed the name of the yellow fever mosquito *Aëdes (Stegomyia) fasciata* to *aegypti* without stating his reasons for doing so. In this connection, my attention has been drawn by Dr. Evans to a letter from Mr. Hopkins, in which he points out that Gough (1914) had already identified *Culex aegypti* L., as *Ochlerotatus caspius* Pallas (*dorsalis* Gough nec. Meigen). In doing so, Gough says: 'This is one of the commonest Culicine mosquitos in our collection, and as Linné's description of *Culex aegypti* fits it very well, there can remain very little doubt that it is really the same species, especially as there appears to be no other common Egyptian species fitting the description.'

This difference of opinion as to the identity of *Culex aegypti* calls for a re-examination of Linné's description, in order to see which of the two species it best fits. This seems to be all the more necessary when it is remembered that the name of this important mosquito has now been changed no less than three times, thereby introducing endless confusion into the medical literature of yellow fever, and there seems no reason to suppose that the name-hunter may not yet find an even older one than that of *aegypti.* The following is Linné's description and a literal translation of it kindly given me by Professor Mountford.

> '*Culex aegypti* articulationibus candidis. Magnitudo Culicis vulgaris Linn. Syst. N. I. Color ex fusco canus. Crura cana cum annulis candidis, parvis, circa articulationes et in articulis. Puncta candida ad marginem dorsi in corpore sub alis utrinque, plura, longitudinaliter sita. Annulus candidus ad basin thoracicis, inter illum et corpus. Linea candida perpendicularis juxta oculos, utrinque una parva. Locus, Aegyptus, Culice communi rarior.'
>
> '*Culex aegypti* with white articulations. The size of the common gnat. *Colour grey from dusky* (*tawny shading into grey*). *Legs grey with white rings, small ones about* (*around*) *the articulations and in the joints.* White spots on the edge of the back on the body, beneath the wings on each side, several of them, placed longitudinally. One white ring at the base of the thorax between it and the body. A white perpendicular line near the eyes, on each side a single small one. Place: Egypt, rarer than the common gnat.'

I have italicised the sentences which, in my opinion, are the most important in arriving at a conclusion as to the identity of *aegypti.* I will now take each part of the description and see whether it would apply either to *O. caspius* or *S. fasciata.* In making this comparison I have been able to examine some fresh specimens of *caspius* presented by Dr. H. H. Salem, Department of Parasitology, University of Cairo, Egypt.

Colour. 'Grey from dusky (tawny shading into grey).' This short terse description of the general colour fits *caspius* exactly. By no stretch of imagination can *fasciata* be said to be a 'grey from dusky' mosquito.

Legs. 'Grey with white rings, small ones about (around) the articulations and in the joints.' Here again this description of the colour of the legs fits *caspius* rather than *fasciata,* the legs of which are black, or dark brown with white apical bands.

Thorax. 'White spots at the edge of the back on the body, beneath the wings on each side, several of them placed longitudinally.' Given a somewhat wide latitude of interpretation of this description of the thorax, it might apply to both species, but, if anything, more to *caspius* than *fasciata.* In *caspius* almost the whole of the mesopleuron is covered with creamy scales, and in rubbed specimens they would appear as spots arranged longitudinally. In *fasciata* there are about six silvery spots on the mesopleuron well separated and arranged in the form of a triangle.

Abdomen. 'A white ring at the base of the thorax, between it and the body.' In *caspius* the first abdominal tergum projects as usual, and has a posterior band of creamy scales; this appearance suggests a white band between the thorax and abdomen. In *fasciata* the silvery scutellum may also be described as a white ring in the same situation.

Head. 'A white perpendicular line near the eyes, on each side a single small one.' This again would apply to the head markings of both species. In *caspius* there is a broad median area of creamy scales and a small creamy area near each eye. In *fasciata* the silvery markings are similarly arranged but are narrower.

It will be noted then that the description of *aegypti* would in the main apply to both species. The general colour and that of the legs, however, definitely rules out *S. fasciata.* Dr. Evans concurs in this opinion. This being the case, it is much to be regretted that due consideration was not given to Gough's opinion before the name of the yellow fever mosquito was changed to *aegypti.* I consider that the name *aegypti* should be dropped, and that the yellow fever mosquito be known in future as *Aëdes* (*Stegomyia*) *fasciata.* I have already pointed out in Part 2 of my book, 'Insects, Ticks, Mites, etc.' (1931), that the name *argenteus* Poiret is also best dropped.

W. S. PATTON.

21 *February,* 1933.

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A Further Note on the Increase of *P. malariae* in Freetown, Sierra Leone. By R. M. GORDON, M.D., and T. H. DAVEY, M.B.

A Yellow Fever Protection Test in Mice by Intracerebral Injection. By MAX THEILER.

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Studies on the Factors that may Influence the Transmission of the Polymorphic Trypanosomes by Tsetse. I.—A Review of Existing Knowledge on this subject, with some General Observations. By H. LYNDHURST DUKE, O.B E., M.D., Sc.D.

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Miscellanea.

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Sleeping sickness was first diagnosed in Kahama in 1928, and the epidemic reached its height in 1929. As the two Districts are contiguous, there is a constant movement of natives between them, and cases began to appear in Kibondo District in 1930, when 55 cases were diagnosed. In 1931, a further 408 cases occurred, the number of cases increasing markedly during the months September to December.

The District was visited in February and March, 1932, while the epidemic was still on the increase, and when, it was to be supposed, the trypanosomes were still at their most resistant stage to human serum; and the opportunity was taken to examine experimentally the resistance to human serum of a large number of fresh strains of *T. rhodesiense*.

The tsetse-fly present was *G. morsitans*, and all the strains examined showed posterior-nuclear forms in rats, with the exception of two, Nos. 16 and 41. Clinically also the disease was of the Rhodesian type.

The incubation periods of the strains differed greatly, varying from 3 days to 30 days, with an average of a little over 8 days. The strains on first isolation were also not very virulent to rats.

EXPERIMENTAL

The technique followed in the following experiments was identical with that described by Yorke, Adams and Murgatroyd (1930). The temperature was maintained at 37° C. for the 12 hours during the daytime; but, as the incubator had no regulating mechanism, the lamp had to be removed for the night, and the temperature fell gradually during the next 12 hours to that of the surroundings—about 21° C.

Rats were usually sub-inoculated with the trypanosome-serum mixture at the end of 24 hours, to see if the absence of trypanosomes, as shown by microscopical examination, really meant that the trypanosomes had been killed by the serum. All experiments were fully controlled by putting up a tube of goat serum and trypanosome suspension; but for the sake of brevity the controls have been left out of the tables.

The results of the examination of 64 strains, all tested in the 1st rat passage from man, are shown in Table I.

Showing the action *in vitro* at 37° C. of human serum on *T. rhodesiense* in the 1st rat passage

Date	Strain	Rat		Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
17.2.32	2	4	13	1/3	H.F.	80	96	62	19	12	4	0	3	**Rat + 5 days**
				1/3	(2)		78	64	24	21	10	13	9	**Rat + 7-10 days**
21.2.32	3	8	17	2/3	H F.	96	78	50	13	14	15	8	6	—
				2/3	(4)		106	45	17	19	7	7	0	—
21.2.32	5	3	12	2/3	H.F.	72	77	54	48	40	19	16	8	—
				2/3	(5)		98	92	73	53	35	44	35	**Rat + 6 days**
21.2.32	6	4	12	1/2	(5)	92	121	76	28	21	12	16	5	—
				1/2	(6)		132	83	23	13	16	10	4	—
21.2.32	17	5	6	pure	(4)	32	54	54	38	26	26	24	18	Rat + 6 days
				,,	(7)		21	47	35	37	30	40	11	—
29.2.32	9	6	20	1/12	H.F	68	42	15	11	12	8	1	2	Rat + 7 days
29.2.32	11	5	16	1/10	H F.	108	114	53	27	26	12	9	9	Rat + 5 days
29.2.32	12	8	16	2/3	H F.	48	56	56	21	1	0	0	1	Rat Neg. 36 days
29.2.32	15	7	15	1/2	H F.	72	48	21	1	7	6	5	3	Rat + 5 days
					(8)		58	53	6	7	0	1	1	—
29.2.32	16	7	15	2/3	(8)	20	24	19	18	17	21	16	10	Rat + 5 days
3.3.32	8	10	23	2/3	H.F.	36	28	29	27	30	24	26	6	Rat + 6 days
3.3.32	10	12	20	1/4	H F.	76	33	32	40	26	26	8	10	Rat + 7 days
					(9)		28	33	22	23	18	12	13	—
3.3.32	19	8	16	1/5	H F	72	40	36	14	7	11	7	3	Rat + 6 days
3.3.32	20	5	16	1/4	H F.	80	64	45	41	39	40	31	17	Rat + 15 days

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1925	Mackay, E. K.
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1926	Fowler, H. P.
1926	Hawe, A. J.
1926	Lennox, D.
1926	Mackay, A. G.
1926	Mackay, D. M.
1926	McLean, N.
1926	MacSweeney, M.

Date of Diploma	
1926	Oppenheimer, F.
1926	Skan, D. A.
1926	Talib, S. A.
1926	Turnbull, N. S.
1927	Allen, C. P.
1927	Austin, T. A.
1927	Besson, W. W.
1927	Dunlop, G. A.
1927	Earl, J. C. St. G.
1927	Hamilton, J.
1927	Harkness, J.
1927	Hay, R.
1927	Hyslop, Kathleen M.

Date of Diploma	
1927	Labuschagne, P. N. H.
1927	McCon, C. F.
1927	Macdonald, J.
1927	Mitchell, Winifred H.
1927	Murray, A. J.
1927	Nevin, H. M.
1927	Nixon, R.
1927	Ormiston, W. S.
1927	Robertson, A.
1927	Walkingshaw, R.
1928	Bilimoria, J. D.
1928	Blakemore, W. L.
1928	Choudari, K. V. R.
1928	Dhar, K. K.
1928	Evans, R. R.
1928	Holmes, W. E.
1928	Laird, W. F.
1928	Maclay, W. S.
1928	Miller, H. V. R.
1928	Morley, A. H.
1928	Pearson, G. H.
1928	Pottinger, J. H.
1928	Sanderson, I.
1928	Sivalingam, S.
1928	Wilkinson, S. A.
1929	Askari, S. W. H.
1929	Drury, G. D
1929	Fraser, N. D.
1929	Halawani, A.
1929	Hilmy, I. S.
1929	Innes, J. A. L.
1929	Lawrence, H. S.
1929	Nixon, R.
1929	Ramdeholl, C.
1929	Setna, H. M.
1930	Anderson, R. E.
1930	Baxter, G. R.
1930	Booker, C. G.
1930	Bullen, W. A.
1930	Chen, T. T. M.
1930	Dobbin, J. H.
1930	Heatley, R. A.
1930	Krishna, R.
1930	Latham, C. N.
1930	McHutchison, G. B.
1930	McMahon, J. E.
1930	Menon, E. S. R.
1930	Reid, A.
1930	Row, C. K.
1930	Wilson, T.
1931	Camps, F. E.
1931	Gauld, E. R.
1931	Loewenthal, L. A.
1931	Mendis, J. E. D
1931	Moir, K. T.
1931	O'Driscoll, F.
1931	Salah, el Din, M.
1931	Sung, J. Koh-si
1931	Verghese, G.
1932	Bowers, G. P. F.
1932	Cathcart, A.
1932	Kurivila, T. K.
1932	Middleton, I. C.
1932	Spreadbury, H. J. H.
1933	Bando, F.
1933	Banker, S. S.
1933	Lowe, Greta
1933	Onwu, S. E.
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1933	Williams, E. G.
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>
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THE ACTION OF HUMAN SERUM *IN VITRO* ON SIXTY-FOUR RECENTLY ISOLATED STRAINS OF *T. RHODESIENSE*

BY

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(*Received for publication* 13 *February*, 1933)

INTRODUCTION

Following the publication by Warrington Yorke, Adams and Murgatroyd (1929) of a technique by which the trypanocidal action of various sera could be examined *in vitro* at 37° C., these workers (1930) published their observations of the action of human sera on various old laboratory strains of *T. gambiense*, *T. equiperdum*, their 'Sherifuri K' strain and *T. congolense*. They showed that, while *T. gambiense* was completely resistant *in vitro* at 37° C. to human serum for 24 hours, *T. equiperdum*, *T. congolense* and *T. rhodesiense* were invariably killed within 2 hours; but their 'Sherifuri K' strain, which they believed to be *T. rhodesiense* (comparatively recently isolated from man) was only killed by human serum after 8 hours. On the basis of this work, they elaborated their hypothesis of the relationship between *T. brucei* and *T. rhodesiense*, i.e., that *T. rhodesiense* is synonymous with *T. brucei* which had acquired a resistance to human serum and so became capable of infecting man. One of the conditions which might lead to a strain of *T. brucei*, normally susceptible to the trypanocidal action of human serum, becoming established in man, might be the presence of individuals in fly bush whose sera showed a diminished or complete absence of trypanolytic power; these individuals, becoming infected with a game strain of *T. brucei* and not overcoming the infection, might establish a strain resistant to normal human serum, and so initiate the spread of sleeping sickness—i.e., a *T. rhodesiense* infection.

THE STRAINS

Kibondo District, where the strains of *T. rhodesiense* used in this investigation originated, is in the north-west of Tanganyika Territory, and on its eastern boundary lies Kahama District.

Sleeping sickness was first diagnosed in Kahama in 1928, and the epidemic reached its height in 1929. As the two Districts are contiguous, there is a constant movement of natives between them, and cases began to appear in Kibondo District in 1930, when 55 cases were diagnosed. In 1931, a further 408 cases occurred, the number of cases increasing markedly during the months September to December.

The District was visited in February and March, 1932, while the epidemic was still on the increase, and when, it was to be supposed, the trypanosomes were still at their most resistant stage to human serum ; and the opportunity was taken to examine experimentally the resistance to human serum of a large number of fresh strains of *T. rhodesiense.*

The tsetse-fly present was *G. morsitans*, and all the strains examined showed posterior-nuclear forms in rats, with the exception of two, Nos. 16 and 41. Clinically also the disease was of the Rhodesian type.

The incubation periods of the strains differed greatly, varying from 3 days to 30 days, with an average of a little over 8 days. The strains on first isolation were also not very virulent to rats.

EXPERIMENTAL

The technique followed in the following experiments was identical with that described by Yorke, Adams and Murgatroyd (1930). The temperature was maintained at 37° C. for the 12 hours during the daytime ; but, as the incubator had no regulating mechanism, the lamp had to be removed for the night, and the temperature fell gradually during the next 12 hours to that of the surroundings—about 21° C.

Rats were usually sub-inoculated with the trypanosome-serum mixture at the end of 24 hours, to see if the absence of trypanosomes, as shown by microscopical examination, really meant that the trypanosomes had been killed by the serum. All experiments were fully controlled by putting up a tube of goat serum and trypanosome suspension ; but for the sake of brevity the controls have been left out of the tables.

The results of the examination of 64 strains, all tested in the 1st rat passage from man, are shown in Table I.

TABLE I

Showing the action *in vitro* at 37° C. of human serum on *T. rhodesiense* in the 1st rat passage

Date	Strain	RAT		Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
17.2.32	2	4	13	1/3	H.F.	80	96	62	19	12	4	0	3	Rat + 5 days
				1/3	(2)		78	64	24	21	10	13	9	Rat + 7-10 days
21.2.32	3	8	17	2/3	H.F.	96	78	50	13	14	15	8	6	—
				2/3	(4)		106	45	17	19	7	7	0	—
21.2.32	5	3	12	2 3	H.F.	72	77	54	48	40	19	16	8	—
				2/3	(5)		98	92	73	53	35	44	35	Rat + 6 days
21.2.32	6	4	12	1/2	(5)	92	121	76	28	21	12	16	5	—
				1/2	(6)		132	83	23	13	16	10	4	—
21.2.32	17	5	6	pure	(4)	32	54	54	38	26	26	24	18	Rat + 6 days
				,,	(7)		21	47	35	37	30	40	11	—
29.2.32	9	6	20	1/12	H.F.	68	42	15	11	12	8	1	2	Rat + 7 days
29.2.32	11	5	16	1/10	H.F.	108	114	53	27	26	12	9	9	Rat + 5 days
29.2.32	12	8	16	2/3	H.F.	48	56	56	21	1	0	0	1	Rat Neg. 36 days
29.2.32	15	7	15	1/2	H.F.	72	48	21	1	7	6	5	3	Rat + 5 days
					(8)		58	53	6	7	0	1	1	—
29.2.32	16	7	15	2/3	(8)	20	24	19	18	17	21	16	10	Rat + 5 days
3.3.32	8	10	23	2/3	H.F.	36	28	29	27	30	24	26	6	Rat + 6 days
3.3.32	10	12	20	1/4	H F.	76	33	32	40	26	26	8	10	Rat + 7 days
					(9)		28	33	22	23	18	12	13	—
3.3.32	19	8	16	1/5	H.F.	72	40	36	14	7	11	7	3	Rat + 6 days
3.3.32	20	5	16	1/4	H.F.	80	64	45	41	39	40	31	17	Rat + 15 days

TABLE I—*Continued*

Date	Strain	Rat		Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
6.3.32	22	7	18	1/3	H.F.	72	96	88	21	4	6	1	1	Rat + 12 days
					(12)		90	56	7	7	5	1	0	—
					(10)		84	24	5	3	0	1	0	—
					(11)		80	37	6	2	1	0	0	Rat Neg. 30 days
10.3.32	43	17	28	1/9	(4)	88	94	90	54	31	24	11	6	—
10.3.32	46	9	20	1/10	(4)	84	74	56	48	19	34	11	13	—
10.3.32	48	5	17	1/8	(4)	148	112	84	58	24	33	12	3	—
10.3.32	51	5	13	1/12	(4)	108	100	51	52	32	31	13	3	Rat + 10 days
12.3.32	47	18-19	22	1/11	(8)	60	13	6	6	4	5	—	4	—
12.3.32	49	6	19	1/6·5	(8)	20	54	24	3	2	2	—	2	—
					(16)		5	3	1	0	1	—	0	—
12.3.32	50	5	15	1/16	(8)	104	48	40	22	23	18	—	12	—
12.3.32	52	11-12	15	1/5	(8)	96	92	26	2	2	2	—	2	—
12.3.32	54	5	15	1/28	(8)	104	75	92	62	31	28	—	16	—
					(16)		80	37	22	20	10	—	5	—
30.3.32	31	7	31	1/16	H.F.	92	120	56	30	24	—	11	16	—
30.3.32	60	5	22	1/7	H.F.	64	78	16	1	0	—	0	1	Rat + 5 days
30.3.32	62	5-8	19	1/8	H.F.	80	116	64	25	26	—	4	10	—
30.3.32	63	10	19	1/2	H.F.	48	56	28	12	11	—	2	7	—
					(20)		74	8	2	3	—	2	2	—
5.4.32	61	3	25	1/3	H.F.	20	44	11	1	3	0	—	1	Rat + 8 days
23.7.31	Paulo	11	21	—	H.F.	110	—	75	44	—	35	16	15	Rat +
17.2.32	4	5	3	1/9	(2)	120	45	8	4	2	4	1	0	Rat + 7-10 days
					(3)		32	8	3	0	0	—	0	Rat + 7-10 days

TABLE I—*Continued*

Date	Strain	RAT		Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
29·2·32	13	6	16	1/7	H.F.	64	56	67	13	8	10	4	0	Rat + 8 days
3·3·32	21	8–11	15	1/6	(9)	84	35	40	17	1	0	1	0	Rat + 6 days
3·3·32	25	4-7	11	1/6	H.F.	108	78	40	8	3	1	3	0	Rat—eaten by others
15·3·32	55	6	18	1/6	H.F.	62	66	31	14	8	9	8	—	—
					(17)		31	20	2	2	3	3	—	—
					(18) S.S.P36		18	10	10	10	11	5	—	—
15·3·32	56	6	18	1/18	H.F.	84	80	52	46	36	27	20	—	—
15·3·32	23	13	27	1/2	H.F.	40	54	39	23	12	8	12	—	—
15·3·32	28	7	17	1/4	H.F.	32	40	32	19	12	12	10	—	—
15·3·32	29	7	19	1/3	H.F.	28	60	46	15	10	8	6	—	—
15·3·32	34	3	13	1/12	(17)	148	160	66	1	1	2	2	—	—
20·3·32	33	7	20	1/2	(19)	64	41	19	10	4	5	2	—	—
20·3·32	35	4	17	1/6	(19)	72	68	53	11	8	2	4	—	—
20·3·32	39	4	13	1/5	(19)	64	59	49	28	18	14	19	—	—
					(16)		21	19	3	1	0	0	—	—
20·3·32	59	5	12	1/20	(19)	92	38	17	12	13	5	3	—	—
20·3·32	42	30	39	1/8·5	(19)	80	18	8	4	3	2	2	—	—
					(16)		6	3	0	1	0	1	—	—
1·9·32	66	7	16	1/6	H.F.	92	76	72	7	6	9	5	0	Rat + 4 days
					Baboon		88	40	4	0	0	0	1	Rat Neg. 30 days
12·3·32	53	5	15	1/13	(8)	92	47	42	6	2	3	—	0	—
20·3·32	44	24	33	—	(19)	96	74	32	8	9	4	0	—	—

TABLE I—*Continued*

Date	Strain	Rat		Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
5.4.32	64	8	24	1/12	H.F.	60	76	7	0	2	2	—	0	Rat + 8 days
15.4.32	38	7	39	1/9·5	H.F.	68	18	4	0	0	1	—	0	Rat + 5 days
17.2.32	1	8	14	1/8	H.F.	72	82	64	8	2	0	0	0	—
					(1)		98	28	0	0	—	—	0	—
6.3.32	27	4-6	13	pure	(12)	16	7	5	1	5	1	0	0	—
				„	(13)		10	8	1	1	0	0	0	Rat Neg. 30 days
				„	(14)		8	5	5	4	3	0	0	—
				„	Goat		3	3	5	4	3	1	0	Rat + 15 days
5.4.32	45	21	46	1/4	H.F.	40	44	12	2	5	0	—	0	Rat + 8 days
30.3.32	36	9	24	pure	H.F.	10	10	6	3	0	—	0	0	Rat + 10 days
30.3.32	37	6	23	1/5	H.F.	52	46	21	2	0	—	0	0	Rat Neg. 36 days
5.4.32	58	5	30	1/20	H.F.	52	27	1	1	0	0	—	0	Rat + 9 days
15.4.32	57	5	41	1/4	H.F.	60	42	15	2	0	0	—	0	Rat + 8 days
5.9.32	67	12	20	1/6	H.F.	116	144	24	5	0	0	0	0	Rat + 7 days
					Baboon		124	48	1	2	1	1	0	Rat Neg. 26 days
10.3.32	18	9	24	1/3	(4)	84	96	7	*0	—	—	—	—	*Rat inoc. + 10 days
15.4.32	32	17-20	47	1/7	H.F.	100	60	9	0	0	0	—	0	Rat + 16 days
15.4.32	65	8	33	1/6	H.F.	88	52	14	0	0	0	—	0	Rat + 8 days
10.3.32	24	6-9	20	1/13	(4)	88	96	*10	—	—	—	—	—	*Rat inoc. Died 16th day
					(15)		76	*0	—	—	—	—	—	*Rat inoc. + 9 days
5.4.32	41	15	55	pure	H.F.	10	1	0	0	0	0	—	0	Rat + 11 days
5.4.32	26	9	44	1/8	H.F.	88	58	7	2	0	0	—	0	Rat Neg. 30 days
					(21)		46	0	0	0	0	—	0	Rat + 7 days

The above table may be summarized as follows :—

Living trypanosomes visible up to	Total number of strains	Trypanosome-serum mixture sub inoculated into rats at 24 hours		
		Number of strains sub-inoculated	Number of rats infected	Strains not infecting
24 hours	30	17	16	No. 12
12 "	16	4	4	—
8 "	4	2	2	—
6 "	3	2	1	No. 27
4 "	5	5	4	No. 37
2 "	3	3	3	—
1 "	3	3	3	—
Total ...	64	36	33	3

The 11 strains tested on March 15th and 20th could only be examined up to 12 hours, and no rats were available for sub-inoculation ; but so many trypanosomes were still alive at the end of 12 hours that there is no doubt that the majority of these strains would have infected rats at the end of 24 hours.

Three strains showed no living trypanosomes in the fluid after 2 hours, and 3 strains none after 1 hour, and yet all these 6 tubes were infective to rats, with incubation periods varying from 7 to 16 days.

Strain No. 12 is of interest in the fact that, although a living trypanosome was seen at 24 hours, the others in the serum were so weakened that they were unable to infect the rat.

It is therefore seen that, using this technique, the fact that trypanosomes are no longer seen in the fluid at any given time does not necessarily mean that they have all been killed ; this can only be proved by inoculating rats with the mixture, and seeing whether they become infected or not. Using a different technique, this had already been noted by Levaditi and Mutermilch (1909) and Kligler (1931).

Neither can the susceptibility of a strain to serum always be judged by the length of time living trypanosomes are visible—cf., for example, strains No. 66 and No. 67, tested against both human and baboon serum, in which trypanosomes were visible in the baboon serum longer than in the human ; cf. also strains Nos. 4 and 26.

It will be noted that in a large number of the strains tested there was a sudden drop, at about 4 hours, in the number of trypanosomes visible under the microscope. This might suggest that, during the development of the trypanosome in the 1st rat, a large number of serum-sensitive variants had arisen ; and that the subsequent infection of the rat, inoculated at 24 hours, might be due to the survival of a few serum-resistant trypanosomes, the numbers of these present in some cases being too few to be seen microscopically.

Without going into percentage figures, it may be said that in *T. rhodesiense* newly isolated, i.e., in the 1st rat passage, the majority of the strains tested were resistant to human serum.

DETAILED EXAMINATION OF 17 STRAINS

The last 17 strains examined were then repeatedly sub-inoculated into rats, and their resistance to human serum examined at each rat passage. The results are embodied in Table II.

TABLE II

Showing the action *in vitro* at 37° C. of the human serum on consecutive rat passages of *T. rhodesiense*

Date	No. of days since strain isolated	Rat			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 58. 5.4.32	M. Aet. 30	25 yrs. 1st	5	30	1/20	H.F.	52	27	1	1	0	0	—	0	Rat + 9 days
21.4.32	46	2nd	5	17	1/20	H.F.	136	104	18	0	0	0*	—	—	*At 8 hours, rat inoc. Rat + 7 days
10.5.32	65	3rd	4	14	1/22	H.F.	100	44	5	0	0	—	0	0	Rat Neg. 30 ,,
26.5.32	81	4th	4	17	1/45	H.F.	112	76	0	0*	—	—	—	—	*Tube broken, rat inoc. 0·2 c.c. Rat Neg. 27 days
9.6.32	95	5th	5	15	1/26	H.F.	72	40	8	0	0	—	0	0	Rat + 6 ,,
17.6.32	103	6th	4	9	1/12	H.F.	80	88	33	3	0	0	0	0	Rat + 5 ,,
27.6.32	113	7th	4	11	1/20	H.F.	128	72	2	0	0	—	0	0	Rat + 6 ,,
13.7.32	129	8th	5	17	1/28	H.F.	124	88	24	1	0	0	0	0	Rat + 5 ,,
25.7.32	141	9th	4	13	1/24	H.F.	92	32	17	0	0	—	0	0	Rat + 7 ,,
6.8.32	153	10th	4	13	1/24	H.F.	80	60	11	0	0	—	0	0	Rat + 4 ,,
						Baboon		38	12	0	0	—	0	0	Rat Neg. 29 ,,
19.8.32	166	11th	5	14	1/8	H.F.	92	76	80	3	0	0	0	0	Rat + 10 ,,
1.9.32	174	12th	4	9	1/44	H.F.	96	92	22	3	2	1	0	0	Rat Neg. 30 ,,
—		13th	4	13	Rat died in	13 days									
21.9.32	199	14th	5	8	1/8	H.F.	88	56	13	6	1	0	0	0	Rat Neg. 38 ,,
30.9.32	208	15th	4	10	1/16	H.F.	88	68	40	2	0	—	0	0	Rat + 7 ,,
STRAIN 62. 30.3.32	F. Aet. 40 19	yrs. Ill 1st	2 months. 5-8	19	1/8	H.F.	80	116	64	25	26	—	4	10	—
29.4.32	49	2nd	6	31	1/10	H.F.	72	46	17	3	2	0	0	0	Rat Neg. 30 days
10.5.32	60	3rd	7	14	1/14	H.F.	76	38	5	0	0	—	0	0	Rat Neg. 30 ,,
26.5.32	76	4th	5	17	1/8	H.F.	96	96	46	18	15	2	0	1	Rat Neg. 27 ,,
—	—	5th	5	14	Rat died in	14 days									

TABLE II—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 62.— 22.6.32	*Continued* 103	6th	4	14	1/12	H.F.	92	72	32	10	5	0	0	0	Rat Neg. 29 days
11.7.32	122	7th	4	20	1/14	H.F.	72	46	0	0	0	—	—	0	Rat Neg. 29 "
21.7.32	132	8th	4	11	1/6·5	H.F.	128	108	56	0	0	—	0	0	Rat Neg. 27 "
—	—	9th	4	15	Rat died in	14 days									
15.8.32	157	10th	7	12	1/28	H.F.	76	44	0	0	0	—	0	0	Rat + 9 "
24.8.32	166	11th	3	10	1/10	H.F.	116	80	8	0	0	—	0	0	Rat Neg. 28 "
1.9.32	174	12th	4	9	1/4	H.F.	96	92	22	3	2	1	0	0	Rat Neg. 30 "
21.9.32	194	13th	4	21	1/10	H.F.	104	72	12	0	0	—	0	0	Rat + 5 "
STRAIN 63. 30.3.32	M. Aet. 19	50 yrs. 1st	Ill 2 months 10	19	1/2	H.F.	48	56	28	12	11	—	2	7	—
						(20)		74	8	2	3	—	2	2	—
21.4.32	41	2nd	6	23	1/11·5	H.F.	76	64	9	0	0	0*	—	—	*At 8 hrs. rat inoc. Rat Neg. 30 days
20.5.32	70	3rd	4	30	1/10	H.F.	64	32	7	0	0	—	0	0	Rat + 6 "
7.6.32	88	4th	5	19	1/18	H.F.	52	32	20	0	0	0	—	0	Rat Neg. 32 "
17.6.32	98	5th	5	11	1/4	H.F.	64	108	20	1	0	0	0	0	Rat + 6 "
27.6.32	108	6th	4	11	1/16	H.F.	68	48	18	0	0	—	0	0	Rat Neg. 31 "
11.7.32	122	7th	5	15	1/10	H.F.	76	56	3 hrs. 3	0	0	—	—	0	Rat Neg. 29 "
21.7.32	132	8th	4	11	1/3·5	H.F.	72	48	36	0	0	—	0	0	Rat Neg. 27 "
4.8.32	146	9th	4	15	1/8	H.F.	88	60	18	0	0	—	0	0	Rat + 6 "
19.8.32	161	10th	7	16	1/10	H.F.	88	80	36	2	0	0	0	0	Rat Neg. 31 "
1.9.32	174	11th	4	14	1/15	H.F.	136	142	44	4	1	1	0	0	Rat Neg. 30 "
21.9.32	194	12th	4	21	1/10	H.F.	84	68	36	4	1	1	0	0	Rat + 6 "

TABLE II—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 65. 15.4.32	M. Aet. 33	30 yrs. 1st	Ill 3 months 8	33	1/6	H.F.	88	52	14	0	0	0	—	0	Rat + 8 days
7.5.32	55	2nd	6	23	1/15·5	H.F.	80	96	28	0	0	0	—	0	Rat Neg. 30 ,,
20.5.32	68	3rd	5	14	1/16	H.F.	88	84	14	0	0	—	0	0	Rat Neg. 31 ,,
9.6.32	88	4th	5	21	1/16	H.F.	80	100	22	2	0	0	0	0	Rat Neg. 30 ,,
22.6.32	101	5th	4	14	1/12	H.F.	76	68	24	2	0	0	0	0	Rat Neg. 29 ,,
11.7.32	120	6th	6	20	1/9	H.F.	92	76	3 hrs. 3	0	0	—	—	0	Rat + 13 ,,
21.7.32	130	7th	4	11	1/14	H.F.	72	116	32	0	0	—	0	0	Rat + 6 ,,
4.8.32	144	8th	4	15	1/6	H.F.	80	56	5	0	0	—	0	0	Rat Neg. 31 ,,
15.8.32	155	9th	7	12	1/5	H.F.	64	60	19	2	0	0	0	0	Rat Neg. 35 ,,
27.8.32	167	10th	4	13	1/20	H.F.	72	72	32	0	0	—	0	0	Rat + 7 ,,
9.9.32	180	11th	4	14	1/5	H.F.	80	84	44	7	0	0	0	0	Rat Neg. 31 ,,
21.9.32	192	12th	4	13	1/20	H.F.	88	84	32	1	0	0	0	0	Rat + 5 ,,
STRAIN 57. 15.4.32	M. Aet. 41	30 yrs. 1st	5	41	1/4	H.F.	60	42	15	2	0	0	—	0	Rat + 8 days
7.5.32	63	2nd	3	23	1/36	H.F.	184	30	2	0	0	0	—	0	Rat Neg. 30 ,,
20.5.32	76	3rd	4	14	1/11	H.F.	72	56	24	0	0	—	0	0	Rat + 5 ,,
—	—	4th	6	16		Rat died in 16 days									
14.6.32	101	5th	5	14	1/10	H.F.	84	64	24	0	0	—	0	0	Rat + 6 ,,
27.6.32	114	6th	6	14	1/12	H.F.	124	68	24	1	0	—	0	0	Rat Neg. 31 ,,
13.7.32	130	7th	5	17	1/3·5	H.F.	52	36	17	0	0	—	0	0	Rat + 8 ,,
25.7.32	142	8th	5	13	1/4	H.F.	68	48	5	0	0	—	0	0	Rat + 6 ,,
6.8.32	154	9th	4	13	1/8	H.F.	68	64	12	0	0	—	0	0	Rat Neg. 29 ,,
19.8.32	167	10th	5	14	1/9	H.F.	88	76	60	0	0	—	0	0	Rat + 7 ,,
27.8.32	175	11th	4	9	1/4	H.F.	92	104	32	14	2	0	0	0	Rat + 6 ,,

TABLE II—*Continued*

Date	No. of days since strain isolated	Rat			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per. sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 26. 5.4.32	M. Aet. 44	30 yrs. 1st	Ill 1 month 9	44	1/8	H.F.	88	58	7	2	0	0	—	0	Rat Neg. 30 days
						(21)		46	0	0	0	0	—	0	Rat + 7 "
29.4.32	68	2nd	5	25	1/11	H.F.	88	36	0	0	0	0	—	0	Rat Neg. 30 "
20.5.32	89	3rd	4	24	1/8	H.F.	52	44	38	4	2	1	0	0	Rat + 6 "
9.6.32	109	4th	5	21	1/10	H.F.	80	40	9	0	0	—	0	0	Rat Neg. 30 "
22.6.32	122	5th	4	14	1/20	H.F.	64	44	4	0	0	—	0	0	Rat + 7 "
11.7.32	141	6th	4	20	1/2	H.F.	72	48	0	0	0	—	—	0	Rat Neg. 28 "
21.7.32	151	7th	4	11	1/8	H.F.	64	60	36	1	0	0	0	0	Rat Neg. 27 "
4.8.32	165	8th	4	15	1/4	H.F.	56	34	7	1	0	0	0	0	Rat + 14 "
15.8.32	176	9th	7	12	1/10	H.F.	80	76	7	0	0	—	0	0	Rat Neg. 35 "
24.8.32	185	10th	3	10	1/6	H.F.	72	84	12	0	0	—	0	0	Rat Neg. 28 "
5.9.32	197	11th	3	13	1/16	H.F.	104	84	18	0	0	0	0	0	Rat Neg. 26 "
21.9.32	213	12th	4	17	1/3	H.F.	28	36	9	0	1	0	0	0	Rat Neg. 38 "
30.9.32	—	—	6	10		Blood clotted in pipette									
17.10.32	239	14th	4	18	1/12	H.F.	76	80	18	1	0	0	0	0	Rat Neg. 29 "
31.10.32	253	15th	4	15	1/4	H.F.	72	88	24	0	0	—	0	0	Rat Neg. 60 "
26.11.32	279	16th	9	27	1/4	H.F.	76	104	16	0	0	—	0	0	Rat Neg. 34 "
5.1.33	319	17th	4	41	1/16	H.F.	136	112	5	0	0	—	0	0	—
STRAIN 45. 5.4.32	F. Aet. 46	40 yrs. 1st	21	46	1/4	H.F.	40	44	12	2	5	0	—	0	Rat + 8 days
21.4.32	62	2nd	4	17	1/16	H.F.	84	74	10	0	1	0	—	0	Rat Neg. 30 "
10.5.32	81	3rd	3	20	1/7	H.F.	60	84	32	6	5	1	2	2	Rat + 12 "
7.6.32	109	4th	4	29	1/12	H.F.	112	72	20	2	0	0	—	0	Rat Neg. 30 "
17.6.32	119	5th	3	11	1/20	H.F.	84	80	14	2	0	0	0	0	Rat Neg. 27 "

TABLE II—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 45—*Continued*															
29 6.32	131	6th	5	13	11/2	H.F.	84	76	6	0	0	—	—	0	Rat + 6 days
18.7.32	150	7th	5	20	1/11	H.F.	104	84	52	0	0	—	0	0	Rat + 7 „
29.7.32	161	8th	5	12	1/20	H.F.	76	56	0	0	0	—	0	0	Rat Neg. 26 „
11.8 32	174	9th	4	14	1/3	H.F.	96	84	12	0	0	—	0	0	Rat Neg. 39 „
24.8.32	187	10th	4	14	1/9	H.F.	64	40	8	0	0	—	0	0	Rat Neg. 28 „
5 9 32	199	11th	4	13	1/6	H F.	72	88	25	5	0	0	0	0	Rat Neg. 26 „
21 9.32	215	12th	4	17	1/18	H.F.	88	56	9	1	0	0	0	0	Rat Neg. 38 „
30.9.32	224	13th	4	10	1/8	H.F.	112	100	48	3	1	—	0	0	Rat Neg. 29 „
17.10 32	241	14th	5	18	1/4·5	H.F.	124	172	19	0	0	0	0	0	Rat Neg. 29 „
31.10.32	255	15th	4	15	1/10	H.F.	72	44	28	0	0	—	0	0	Rat Neg. 60 „
26.11.32	281	16th	6	27	1/4	H.F.	28	32	3	0	0	—	0	0	Rat Neg. 34 „
			Rat eaten by companion, strain lost.												
STRAIN 61.	F. Aet.	30 yrs.	Ill 1 month												
5.4.32	25	1st	3	25	1/3	H.F.	20	44	11	1	3	0	—	1	Rat + 9 days
29 4.32	49	2nd	6	25	1/11·5	H.F.	72	100	50	8	5	4	3	2	—
10.5.32	60	3rd	4	14	1/16	H.F.	88	112	14	0	0	—	0	0	Rat + 6 days
26 5.32	76	4th	4	17	1/15	H.F.	76	72	16	2	0	0	—	0	Rat Neg. 27 „
9 6.32	90	5th	5	15	1/14	H.F.	76	88	5	0	0	—	0	0	Rat + 7 „
17 6.32	98	6th	4	9	1/7	H.F.	68	60	36	0	0	—	0	0	Rat + 8 „
27.6.32	108	7th	4	11	1/9	H.F.	72	60	14	0	0	—	0	0	Rat + 6 „
13.7.32	124	8th	5	17	1/6	H.F.	84	44	16	0	0	—	0	0	Rat Neg. 27 „
29.7.32	140	9th	7	17	1/16	H.F.	96	60	2	0	0	—	0	0	Rat Neg. 26 „
11.8.32	153	10th	4	14	2/3	H.F.	8	16	0	0	0	—	0	0	Rat Neg. 39 „
27.8.32	169	11th	4	17	1/2	H.F.	128	60	5	0	1	0	0	0	Rat + 7 „
9.9.32	182	12th	4	14	1/5	H.F.	72	88	52	3	0	0	0	0	Rat + 7 „

TABLE II—*Continued*

Date	No. of days since strain isolated	Rat			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 38. 15·4·32	M. Aet. 39	30 yrs. 1st	Ill 1 month 7	39	1/9·5	H.F.	68	18	4	0	0	1	—	0	Rat + 5 days
7·5·32	61	2nd	3	23	1/17	H.F.	96	76	24	0	0	0	—	0	Rat Neg. 30 "
20·5·32	74	3rd	5	14	1/9	H.F.	52	52	18	1	0	0	0	0	Rat Neg. 31 "
7·6·32	92	4th	5	19	1/32	H.F.	140	96	30	2	2	0	0	0	Rat + 7 "
17·6·32	102	5th	4	11	1/20	H.F.	50	40	5	0	0	—	0	0	Rat Neg. 27 "
29·6·32	114	6th	4	13	1/40	H.F.	84	64	15	0	0	—	—	0	Rat died on 6th day
13·7·32	128	7th	5	15	1/64	H.F.	76	76	8	1	0	0	0	0	Rat Neg. 27 days
25·7·32	140	8th	4	13	1/36	H.F.	56	7	0	0	0	—	0	0	Rat Neg. 30 "
4·8·32	150	9th	4	11	1/28	H.F.	132	52	18	0	0	—	0	0	Rat Neg. 46 "
15·8·32	161	10th	7	12	—	H.F.	132	132	15	1	0	0	0	0	Rat Neg. 35 "
24·8·32	170	11th	4	10	1/20	H.F.	104	32	2	0	0	—	0	0	Rat Neg. 28 "
5·9·32	182	12th	3	13	1/12	H.F.	104	92	16	2	0	1	0	0	Rat + 7 "
STRAIN 60. 30·3·32	M. Aet. 22	20 yrs. 1st	Ill 4 months 5	22	1/7	H.F.	64	78	16	1	0	—	0	1	Rat + 5 days
29·4·32	52	2nd	5	31	1/8	H.F.	64	50	17	0	0	0	—	0	Rat + 6 "
10·5·32	63	3rd	7	14	1/12	H.F.	72	28	4	0	0	—	0	0	Rat Neg. 30 "
26·5·32	79	4th	5	17	1/8	H.F.	88	124	4	2	0	0	—	0	Rat + 5 "
9·6·32	93	5th	5	15	1/12	H.F.	80	96	23	4	4	1	0	0	Rat Neg. 30 "
17·6·32	101	6th	5	9	1/8	H.F.	100	112	76	36	22	5	1	0	Rat Neg. 27 "
29·6·32	113	7th	5	13	1/10	H.F.	92	84	11	1	0	0	—	0	Rat Neg. 29 "
18·7·32	132	8th	5	20	1/14	H.F.	48	48	26	0	0	—	0	0	Rat Neg. 30 "
29·7·32	143	9th	5	12	1/10	H.F.	104	52	10	0	0	—	0	0	Rat + 7 "
11·8·32	156	10th	4	14	1/12	H.F.	76	60	4	0	0	—	0	0	Rat Neg. 39 "
24·8·32	169	11th	4	14	1/7	H.F.	128	112	60	0	0	—	0	0	Rat + 6 "

TABLE II—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 36.	F. Aet.	35 yrs.	Ill 5 days												
30.3.32	24	1st	9	24	pure	H.F.	10	10	6	3	0	—	0	0	Rat + 10 days
7.5.32	62	2nd	8	39	1/14	H.F.	72	72	34	2	0	0	—	0	Rat Neg. 30 "
26.5.32	81	3rd	5	20	1/8	H.F.	84	64	9	0	0	0	—	0	Rat Neg. 27 "
14.6.32	100	4th	6	20	1/10	H.F.	96	42	16	5	0	0	0	0	Rat + 7 "
29.6.32	115	5th	6	16	1/8	H.F.	88	88	14	1	0	0	—	0	Rat Neg. 29 "
13.7.32	129	6th	5	15	1/6	H.F.	84	76	28	21	4	2	1	0	Rat + 4 "
25.7.32	141	7th	4	13	1/14	H.F.	92	64	24	0	0	—	0	0	Rat Neg. 30 "
4.8.32	151	8th	4	11	1/20	H.F.	84	36	5	0	0	—	0	0	Rat Neg. 31 "
15.8.32	162	9th	7	12	1/16	H.F.	60	44	1	0	0	—	0	0	Rat + 6 "
STRAIN 37.	M. Aet.	55 yrs.	Ill 2 months												
30.3.32	23	1st	6	23	1/5	H.F.	52	46	21	2	0	—	0	0	Rat Neg. 36 days
21.4.32	45	2nd	5	23	1/13·5	H.F.	84	80	46	3	1	0	—	0	Rat + 6 "
10.5.32	64	3rd	3	20	1/16	H.F.	84	104	21	0	0	—	0	0	Rat Neg. 30 "
—	—	4th	4	29		Rat died in 29 days									
14.6.32	99	5th	3	8	1/10	H.F.	120	64	40	1	0	0	0	0	Rat Neg. 30 "
27.6.32	112	6th	5	14	1/44	H.F.	92	4	4 3 hrs.	0	0	—	0	0	Rat Neg. 31 "
11.7.32	126	7th	5	15	1/26	H.F.	116	11	0	0	0	—	—	0	Rat + 6 "
21.7.32	136	8th	4	11	1/32	H.F.	112	32	1	0	0	—	0	0	Rat + 9 "
			Rat eaten by companion on 7th day : strain lost.												

TABLE II—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 31. 30.3.32	M. Aet. 31	40 yrs. 1st	7	31	1/16	H.F.	92	120	56	30	24	—	11	16	—
21.4.32	53	2nd	3	23	1/36	H.F.	72	46	8	0	1	0	—	0	Rat + 10 days
7.5.32	69	3rd	4	17	1/32	H.F.	88	52	7	0	0	0	—	0	Rat Neg. 30 "
26.5.32	88	4th	4	20	1/14	H.F.	52	68	8	0	0	0	—	0	Rat Neg. 27 "
9.6.32	102	5th	4	15	1/13	H.F.	88	36	5	1	0	0	0	0	Rat Neg. 30 "
22.6.32	115	6th	5	14	1/9·5	H.F.	52	76	32	1	0	0	0	0	Rat Neg. 29 "
—	—	7th	6	18		Rat died in 18 days.									
21.7.32	144	8th	4	12	1/16	H.F.	96	56	0	0	0	—	0	0	Rat Neg. 27 "
				Rat eaten by companion on 10th day : strain lost.											
STRAIN 32. 15.4.32	F. Aet. 47	40 yrs. 1st	Ill 2 months 117	47	1/7	H.F.	100	60	9	0	0	0	—	0	Rat + 16 days
7.5.32	69	2nd	5	23	1/9	H.F.	96	60	44	1	0	0	—	0	Rat Neg. 30 "
7.6.32	100	3rd	6	32	1/5	H.F.	60	60	32	1	0	0	—	0	Rat Neg. 32 "
22.6.32	115	4th	4	16	1/5	H.F.	96	72	92 3 hrs.	10	2	2	0	0	Rat + 11 "
11.7.32	134	5th	6	20	—	H.F.	44	64	3	1	0	0	—	0	Rat Neg. 29 "
18.7.32	141	6th	4	8	pure	H.F.	32	28	16	5	0	0	0	0	Rat Neg. 30 "
29.7.32	152	7th	5	12	1/8	H.F.	72	80	4	0	0	—	0	0	Rat Neg. 26 "
6.8.32	160	8th	5	9	1/7	H.F.	84	60	44	5	0	0	0	0	Rat Neg. 29 "
—	—	9th	5	14		Rat died in 14 days.									
27.8.32	181	10th	4	9	1/36	H.F.	72	40	5	0	—	—	—	—	Tube dropped
				Rat eaten by companion on 6th day : strain lost.											

TABLE II—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum	Trypanosomes per. sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
STRAIN 64.	F. Aet.	50 yrs.	Ill 3 months												
5.4.32	24	1st	8	24	1/12	H.F.	60	76	7	0	2	2	—	0	Rat + 8 days
29.4.32	48	2nd	5	25	1/3	H.F.	84	104	33	0	0	0	—	0	Rat Neg. 30 „
20.5.32	69	3rd	4	24	1/19	H.F.	92	48	1	0	0	—	0	0	Rat Neg. 31 „
7.6.32	87	4th	5	19	1/32	H.F.	120	76	26	2	0	0	—	0	Rat + 9 „
14.6.32	94	5th	3	8	1/9	H.F.	56	20	7	0	0	—	0	0	Rat Neg. 30 „
27.6.32	107	6th	5	14	1/2	H.F.	40	80	23	3	0	—	0	0	Rat + 10 „
13.7.32	123	7th	5	17	1/21	H.F.	92	56	8	0	0	—	0	0	Rat Neg. 27 „
—	—	8th	4	Rat died in 5 days. Rat sub-inoculated on 4th day and failed to infect.											
STRAIN 41.	M. Aet.	30 yrs.													
5.4.32	55	1st	15	55	pure	H.F.	10	1	0	0	0	0	—	0	Rat + 11 days
21.4.32	71	2nd	5	17	1/10	H.F.	120	36	4	0	0	0	*	*	Rat inoc. at 8 hrs. Rat Neg. 30 days
—	—	3rd	3			Rat died in 12 days									
STRAIN 67.	M. Aet.	40 yrs.	Ill 1¼ months.												
5.9.32	20	1st	12	20	1/6	H.F.	116	144	24	5	0	0	0	0	Rat + 7 days
						Baboon		124	48	1	2	1	1	0	Rat Neg. 26 „
26.11.32 g.-pig. tsetse	102	—	17-18	—	—	H.F.	80	112	20	5	2	3	2	1	—

This table is of peculiar interest in showing how very frequently, when a strain is carried on by syringe, the resistance to human serum disappears as early as the 2nd rat passage, to re-appear in subsequent passages. It would therefore appear that, when any strain is isolated from man, game or tsetse, its resistance to serum should be tested in the 1st rat passage, as subsequent passages might give misleading results (unless a long and consecutive series of passages were tested).

In this connection it is of interest to examine strains Nos. 41 and 67. Strain 41 was sub-inoculated, and, tested on the 2nd rat passage, the resistance to serum had decreased. Strain 67, on the other hand, was transmitted by fly to a guinea-pig, and on the 2nd passage the resistance had markedly increased. The two experiments are not quite comparable, as in one case the 2nd passage was to a rat, while in the other it was to a guinea-pig ; but, as Lester (1932) has shown that a serum-resistant strain of *T. brucei* in a mouse, when sub-inoculated into a guinea-pig, has its serum-resistance decreased, the above result is all the more srtiking.

This experiment with strain 67, slight as it is, would seem to support the observations of Schilling and Schreck (1930) and Lester (1932), that the cyclical passage of a strain through tsetse has a stabilising effect on the trypanosome, not only in regard to virulence and serological properties but also in regard to resistance to human serum.

A few experiments with *T. brucei*, tested on the 1st rat passage after isolation, have shown that the trypanosome is susceptible to human serum, no trypanosomes being seen microscopically after 2 hours, and the rats inoculated at 24 hours not being infected : it is hoped to examine shortly a larger number of strains by this technique.

If it is shown conclusively that, on *T. rhodesiense* being transmitted cyclically through tsetse, it maintains its resistance to human serum on each passage, then a biological method of differentiating *T. rhodesiense* and *T. brucei* would result, as in any area, especially in sleeping sickness areas, those polymorphic trypanosomes recovered from game or fly, which show, when tested *in vitro* at the first rat passage, a resistance to human serum, would be justifiably called *T. rhodesiense.*

TRYPANOCIDAL ACTION OF VARIOUS HUMAN SERA

It was impossible in the time available, to examine the trypanocidal action of many sera from the Kibondo natives ; but the sera of 10 natives, who had come to the out-patient department for treatment of yaws, ulcers, etc., were tested, and they did not differ from the normal (Table I, sera Nos. 1, 2, 3, 5, 6, 7, 9, 10, 11 and 17). In all, 21 sera were examined, without any marked variation in trypanocidal action being observed.

In Table III are shown the results of a further 8 sera tested in 1931, which again shows no serum deviating markedly from the normal.

On two occasions (Table I, strain 55 and Table III, strain 'Kungulu '), serum was taken from a new sleeping sickness case *before treatment*, and its trypanocidal power tested against a heterologous strain. On both occasions they showed similar trypanocidal power to the author's serum.

It was also found that it was not always essential to dilute the rat's blood with Ringer-glucose solution, as with undiluted rat blood added to human serum trypanosomes survived for 24 hours (Table I, strains 17, 27, 36 and 41).

TABLE III

Showing the trypanocidal action of 8 sera tested against a known normal

Date	Strain	RAT			Serum	Trypanosomes per sq. mm. (Thoma-Zeiss)								Remarks
		Passage	Incubation period	No. of days since sub-inoculation		Start	1	2	4	6	8	12	24	
23.7.31	Nyangaraza d/o Kapela	1st	—	7	H.F.	80	—	13	0	—	0	—	0	
7.11.31	Namwelu d/o Mwizagi	1st	9	28	H.F.	60	65	47	20	—	0	—	—	
					(22) D.B.W.		111	78	1	—	0	—	—	
					(23) E.H.A.		99	83	15	—	0	—	—	
					(24) Henry		72	41	2	—	0	—	—	
28.11.31	Mwenda s/o Kafuku	1st	9	22	(25) Nicholas	90	86	49	14	3	0	—	—	
					(26) Rochi		70	28	0	0	—	—	—	
					(27) Emmanul		97	51	1	2	0	—	—	
					(28) Robert		53	27	1	0	0	—	—	
11.10.31	Kungulu s/o Kasyora	2nd	3	10	H.F.	60	35	5	0	—	0	—	—	
					(19)		42	0	0	—	0	—	—	
					(29) Namwelu d/o Mwizagi S.S. case before treatment		24	17	0	—	0	—	—	

SUMMARY

1. During a recent outbreak of sleeping sickness in the Kibondo District of Tanganyika Territory, the action of human serum was tested *in vitro* at 37° C. against 64 strains of *T. rhodesiense* (all in the 1st rat passage). The majority of these strains were shown to be resistant.

2. It was shown that the resistance to human serum was a very variable quality, fluctuating from rat passage to rat passage.

3. In a large number of the 2nd rat passages tested, this resistance to human serum has disappeared, to re-appear later; and it is therefore suggested that in future, when testing any strain, it should always be done in the 1st rat passage, or else over a long and consecutive series of subsequent passages.

4. Following Yorke, Adams and Murgatroyd's technique, it has been shown that it is not justifiable to say a strain is non-effective just because no trypanosomes are visible in the fluid after 2 to 4 hours, but that this must be established by sub-inoculating rats.

5. On the other hand, even though an occasional trypanosome is seen in the fluid at the end of 24 hours, the sub-inoculated rat may not be infected.

6. Twenty-nine human sera (both European and native) were tested for their trypanocidal power, and they were all of the same value.

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HOOKWORM DISEASE IN THE ARAB OF THE MIDDLE EUPHRATES

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The world map which accompanies Darling's description of hookworm disease in the 'Nelson Loose-leaf Medicine' indicates the Middle Euphrates as an area from which no report of the disease has issued. We are herewith reporting our experience with ankylostomiasis in this area.

The course of the Euphrates river may be roughly divided into three parts. The river rises in Turkey, and its first section traverses the valleys of a mountainous region. Its second part sweeps in a wide curve through the Syrian desert across the French mandated country of Syria. The third part approaches the Tigris river, and the two rivers together water the independent Kingdom of Iraq and join to flow into the Persian Gulf.

The Middle Euphrates runs in a south-easterly direction across Syria between the latitudes of 37° and 34° North. It is joined by two streams from the north, the Belikh and the Khabour. The main river and its two tributaries support a large Arab farming population gathered in the mud villages which line their banks. There are also several large towns at points along the rivers, the largest of which is Deir-ez-Zor with a population of 30,000.

On *a priori* grounds, one would expect to find infestation with hookworm in this region. The Arab even in the towns is devoid of a sense of sanitary cleanliness, and the Moslem custom of ablutions leads to the use of the river bank as the community latrine. In the towns, the streets, alleys and courts are also polluted. Most of the town children run barefooted throughout the year, and the feet of the village and farming population are almost uniformly unshod. The soil of the region is light river deposit, and irrigating water is carried to the fields through open ditches. Much of the farmers' work is done in moist soil. The climate of the region is usually mild in winter though occasionally there may be hard frosts and even snow. The summer is very hot and dry.

Hookworm has been reported in Iraq along the Euphrates and Tigris in their lower reaches ; however, it has not been reported nor apparently recognised in the course of the Middle Euphrates. In an analysis of 2,000 consecutive admissions to the American Mission out-patient clinic of Deir-ez-Zor (Hudson and Young, 1931), we reported 28 cases of ankylostomiasis in the series. Since then about 3,400 further patients have passed through the clinic, and among these have been 81 further cases of ankylostomiasis, making a total of 109 clinic patients with hookworm, out of 5,400 clinic admissions.

There were 28 cases in the first 2,000, 5 in the third 1,000, 21 in the fourth 1,000, and 48 in the fifth 1,000. These were all cases in which ova of *Ankylostoma duodenale* were found in the stool. Most of the stools were examined by cultural or centrifugation methods.

That the figure of 109 out of 5,400 does not represent the total amount of hookworm infestation is apparent from the fact that stool examinations are not carried out routinely in the clinic, and that the number per thousand has risen with our increasing awareness of the prevalence of the disease. Our figure of 109 positive cases does not include others in which hookworm was suspected but unconfirmed by stool examination.

In our first series of 28 patients, 18 came from the town of Deir-ez-Zor, but as the number of our bedouin and agricultural patients has increased we have realised that, however common it may be in the towns, its incidence is greater, as might be expected, in the farming villages. For example, out of the last 3,400 clinic admissions, 20 cases have been from the town, and 61 have come from the farm, although the farming communities furnish less than one-third of the total of clinic admissions.

The incidence of the disease in relation to sex shows 16 males and 4 females in 20 town-dwellers, and 47 males out of 61 cases from farms. One may conclude that males both in town and country are about four times as heavily infested as females.

As to age distribution, we find the average age of 20 townspeople to be 23 years, the youngest 2 years old and the oldest 50 years of age. In the country population the average age of 61 cases is 26 years, the youngest being 4 years old and the oldest 60.

Distributing the town-dwelling cases by age decades, we find 5 in the first decade, 4 in the second, 7 in the third, 3 in the fourth, and 1 in the fifth. Thus 80 per cent. are below the age of 30 years.

Of the 61 agricultural cases, 4 are found in the first decade of life, 16 in the second, 25 in the third, 11 in the fourth, 3 in the fifth, and 2 in the sixth. Here 75 per cent. of the cases are below the age of 30, and the greatest infestation is found in the decade from 20 to 30.

We believe this does not give a true picture of the general distribution of the disease, as many children are allowed to suffer from the disease untreated, whereas the young male who is the bread-winner is forced to seek treatment. A hookworm census of the general population would probably show a heavy incidence among children. The clinician in this region comes to suspect a triad of diseases in each bedouin child whom he examines, namely, syphilis (Hudson, 1932), amoebic dysentery and hookworm. Occasionally he finds all three diseases present in the same child.

We have no data upon which to make a conclusive statement as to the degree of infestation of the average patient, as we have not done any worm counts after medication. We have seen a few adults with very severe clinical symptoms, but for the most part it would seem that adult infestations are mild in type, producing gastro-intestinal symptoms without severe anaemia. We suspect that the severe symptoms would be found more commonly in a series of bedouin children.

From the standpoint of geographical distribution, most of our clinic patients naturally have come from Deir-ez-Zor itself and from nearby villages for a distance of 20 or 30 miles up and down the Euphrates. A few, however, have come from distant Euphrates villages, and others have come from farming communities on the Khabour river, from a distance of perhaps 100 miles. We conclude, therefore, that the disease is not limited to the immediate vicinity of Deir-ez-Zor, but is equally prevalent throughout the length of the Middle Euphrates and its two tributaries.

This conclusion is re-enforced by the result of a survey of 306 Arab workmen engaged in excavations near Salihiyeh, 60 miles down the Euphrates from Deir-ez-Zor. These men come from the agricultural communities along the river nearby, and are employed by the Yale University Archaeological Expedition, which is excavating the ancient town and fortress of Doura-Europus. It was through the courtesy of Dr. Clark Hopkins, director of the expedition, that we carried out this survey in the spring of 1933.

None but men and boys are employed in the excavations, and the stools of all but a few of the workmen were examined. One hundred and seventeen out of the 306 examined were found to be harbouring hookworms, an incidence of 38·2 per cent. These stools were examined by the brine flotation method.

The age of the youngest subject was 9 years, and the oldest gave the age of 60. Of the 117 cases in which hookworms were found, 11 were in the first decade of age, 39 were in the second, 28 were in the third, 27 were in the fourth, 5 were in the fifth, 3 were in the sixth, and 4 were in the seventh decade. Naturally, this was a selected group, and its age distribution cannot be applied to the general population. However, it may be noted that two-thirds of the cases were below 30 years of age.

Inasmuch as defaecation by the workmen is promiscuous about the diggings, it is possible that the positive percentage may have been increased by the proximity of the workmen in their gangs, but the season of digging is a short one and the labour turnover considerable, so that this factor may not have much weight. On the other hand, these subjects were presumably healthy, doing hard day labour, so that it is obvious that they do not represent the sick population, which would doubtless contain a greater incidence of hookworm infestation.

One may safely conclude that the incidence of hookworm in the male Arab farming population of the neighbourhood of Salihiyeh is probably 1 in 4, and possibly as great as 1 in 3. There is ground for the suspicion that this incidence of the disease would be found in the male population of the farming communities throughout the length of the Euphrates river and its tributaries in the course of their flow through Syria.

If this is true, the disease constitutes a public health problem of some magnitude for this region of the State of Syria.

Incidentally, in the course of the stool examination of the 306

Arab workmen we found, in addition to hookworm ova, only 17 cases of infestation with *Ascaris lumbricoides* and 2 with *Taenia saginata*. This low incidence of other intestinal parasites is doubtless due to the lack of fresh vegetables and meat in the bedouin farmer's diet. His staple food throughout the year is wheat and Kaffir corn. This low incidence of *Ascaris* and *Taenia* is remarkable, as these parasites are prevalent elsewhere in Syria, especially in the cities. We found (Hudson *et al.*, 1930) a morbidity of 75 per cent. for *Ascaris lumbricoides* in a survey of the children of a school in Aleppo.

SUMMARY

1. One hundred and nine proved cases of hookworm infestation are reported in a series of 5,400 consecutive out-patients in Deir-ez-Zor. Some of the hookworm cases are town-dwellers, but the majority come from farming villages. Many cases of hookworm infestation were doubtless missed in the course of the 5,400 admissions, so that the local incidence of the disease is undoubtedly higher than the above figure would indicate.

2. Males were found to be infested 4 times as frequently as females, and the majority of the patients were under 30 years of age. These clinic statistics give no adequate picture of the amount of childhood infestation, which is undoubtedly heavy.

3. The clinical course of the disease seems in general to be mild, though again full information on this point is lacking, especially among the childhood population.

4. Our clinic series contains patients who came from villages distant from Deir-ez-Zor, thus indicating that the disease is prevalent more or less throughout the length of the Middle Euphrates and its two tributaries in Syria. This surmise is strengthened by the result of a survey of 306 presumably healthy Arabs working at a point on the Euphrates 60 miles east of Deir-ez-Zor. Thirty-eight per cent. of this group were found to be harbouring hookworms.

5. There are good grounds for believing that 1 in 4, or possibly 1 in 3, of the male Arab farming population of the Euphrates basin in Syria harbours hookworms. A more extensive and intensive survey of the hookworm situation in this region is indicated.

6. It seems that the presence of ankylostomiasis has hitherto not

been recognised in the basin of the Middle Euphrates, and this—so far as we are aware—is the first time it has been reported in this region. Along with syphilis and amoebic dysentery, ankylostomiasis constitutes a major public health problem in this part of Syria.

Note.—The authors are indebted to their colleague, Dr. S. S. Crosley, for permission to include her cases of hookworm in this report.

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SOME REMARKS ABOUT THE RÔLE OF TRUE BLOOD-SUCKING *MUSCA* SPECIES AS TRANSMITTERS OF DISEASES

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In his latest publication on the genus *Musca* in this journal, Patton (1932) states in connection with the true blood-sucking species of this genus: 'These species, too, are potential transmitters of pathogenic organisms both mechanically and biologically, but as yet nothing is known of their capabilities in this direction.' However, a certain amount of work has been done on these problems, and, on account of the authoritative manner of the statement, a short note on the true position may be given, as these flies are really interesting and not devoid of practical importance.

Experiments with surra were carried out by Mitzmain, Patel and Nieschulz. Mitzmain (1914) got one positive result by direct transmission from monkey to monkey with 104 specimens, whereas 7 experiments with an unknown number of flies were negative. After an interval of 1 hour, experiments with 18, and after 24 hours, experiments with 32, specimens were negative. With the same species, Patel (in Fletcher, 1916) got positive results after intervals not exceeding 7 minutes. No further details were given. Nieschulz (1927, 1930) obtained only negative results with 103 *Musca crassirostris* in experiments with horses, with 19 specimens with guinea-pigs and rats, and with 100 *Musca inferior* in one experiment with horses.

Anthrax was transmitted by Nieschulz (1929) with *Musca crassirostris* from guinea-pig to guinea-pig. By direct transmission, 1 out of 6 experiments with a total of 11 flies was positive.

Haemorrhagic septicaemia of buffaloes was transmitted by Nieschulz and Kraneveld (1929) through *Musca crassirostris* in experiments with rabbits. Positive results were obtained by direct transmission and after intervals varying between half-an-hour and 24 hours. The number of flies used were by direct transmission 2, after half-an-hour 4, after 1 hour 1, after 3 hours 2, after 6 and 24 hours 4. At each interval 1 positive result was obtained, showing that the capacity of the fly to transmit this disease was quite high.

Thus *Musca crassirostris* has proved to be capable experimentally of transmitting surra (Mitzmain, Patel), anthrax (Nieschulz) and barbone disease of buffaloes (Nieschulz and Kraneveld). As mechanical transmitter of these diseases, its capacity ranges between *Stomoxys* and *Lyperosia*. Being a very common species in the Orient and the Far East, and being not uncommon in South Africa, it is bound to be of some economic importance.

SUMMARY

A review of the work done on the transmission of surra, anthrax and haemorrhagic septicaemia of buffaloes by *Musca crassirostris*, a true blood-sucking *Musca* species, is given.

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A STUDY OF THE BEHAVIOUR OF *T. RHODESIENSE* RECENTLY ISOLATED FROM MAN, IN ANTELOPE AND OTHER AFRICAN GAME ANIMALS

PART I

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CONTENTS

		PAGE
I.	INTRODUCTION	215
II.	HISTORY OF THE STRAINS OF *T. rhodesiense* USED IN THESE EXPERIMENTS	219
III.	SCHEMATIC REPRESENTATION OF THE PASSAGES FROM ANIMAL TO ANIMAL	221
IV.	THE ANTELOPE AND THE HISTORY OF THEIR INFECTION	222
V.	ACCOUNT OF THE TRANSMISSION EXPERIMENTS WITH *G. palpalis* AND *G. morsitans*.	225
VI.	*T. rhodesiense* IN GAME ANIMALS OTHER THAN ANTELOPE	231
VII.	SUMMARY	236

I. INTRODUCTION

Ever since the first recognition, some twenty years ago, of *T. rhodesiense* as a parasite of man, its relation to the two well-known trypanosomes, *T. gambiense* and *T. brucei*, has been a subject of speculation and debate. The range of *T. rhodesiense* extends from the Southern Sudan to Rhodesia, and from the Eastern Congo and Uganda right across Tanganyika Territory. For political reasons, field research into the biology of *T. rhodesiense* has fallen almost exclusively to the lot of British and German investigators, and for a number of years the two schools have held fundamentally different views about the affinities of this trypanosome. Kleine and his colleagues believe *T. rhodesiense* to be a different species from *T. brucei*; on the other hand, Kinghorn and Yorke, and Bruce and his collaborators, have always maintained that these two trypanosomes are zoologically one and the same species, *T. rhodesiense* being merely a variety of *T. brucei* that can infect man. From time to time, observers in the field have inoculated themselves or their associates with strains of *T. brucei* from regions where no human trypanosomiasis was known to exist. In every case the trypanosomes failed to survive in man. Then, in 1917, came Taute's great experiment, which consisted in the inoculation of 129 natives, and 2 Europeans, with strains of *T. brucei* from a region known to be free from any human trypanosome (Taute and Huber, 1919). The Germans saw in this experiment confirmation of their belief that *T. brucei* and *T. rhodesiense* are different species. To their opponents, Taute's experiment was merely additional proof that normal *T. brucei* cannot touch man. At this stage the discussion has remained for a number of years. With the formation of the League of Nations Commission early in 1926, Kleine, after spending some months in the *gambiense* areas of Kavirondo, went to Tanganyika Territory to take up the study of *T. rhodesiense*. As a result of his work at Ikoma, he reached the conclusion that *T. gambiense* and *T. rhodesiense* were but different forms of the same species of trypanosome, and at the same time he reiterated his original opinion that both the human trypanosomes were fundamentally distinct from *T. brucei*. By studying a number of different strains of *T. rhodesiense* freshly isolated from man, Kleine found that some of them behaved like *T. gambiense* and produced chronic infections in laboratory animals and very few posterior nuclear forms. Some of Kleine's results were published in the Final Report of the Commission. In the same report appears a discussion on the prevailing views about *T. rhodesiense* (Duke, 1927), in which it was pointed out that the human inoculation experiments performed up to date related only to a very small number of different strains of *T. brucei*, which certainly appeared to be incapable of infecting either sick or healthy man. What still remained to be done was to expose man to a number of strains of *T. brucei* to see whether different strains differed in this important respect. In the same section some evidence was given, meagre perhaps but quite definite, that *T. rhodesiense* differed from typical *T. brucei* in possessing greater pathogenicity to domestic stock. Corson has since confirmed this experience, one of his strains killing goats in some 4 weeks—a remarkable manifestation of virulence in this hardy animal. Here is a point of some importance. Bruce and his colleagues in Nyasaland found the average duration of disease caused by the human strain of *T. rhodesiense* in 29 goats to be 42 days. One ox inoculated with this same strain lived 134 days and 4 other oxen recovered (Bruce *et al.*, 1915). Hitherto, the only known difference between *T. rhodesiense* and *T. brucei* has been their behaviour in man's blood. If the two trypanosomes are found to differ consistently in their pathogenicity to domestic ruminants, the gap between them widens. This possibility is at present being investigated at the Veterinary Laboratory at Entebbe, where Mr. R. W. M. Mettam is testing some of our strains of *T. rhodesiense* in domestic stock.

For some years past I have held the view that typical *T. gambiense*, mild in all its animal reactions, represents the highest degree of adjustment yet obtained between a trypanosome of the polymorphic group and man. The Eket strains, described years ago by McFie in West Africa, and also some of those recently studied by Taylor in Nigeria, appear to have established relations with man reminiscent of the adaptation of *T. brucei* to the big game in nature. In both these areas the trypanosome has in all probability been dependent on man for long periods of time, during which selection has gradually perfected the adjustment between the parasite and both its vector and its definitive host. Kleine's studies with the Commission led him to link up *T. gambiense* and *T. rhodesiense*, and at the same time confirmed his original views about *T. brucei*. He suggests that the chronic strains found around Ikoma represent a stage in the adaptation of the characteristically fierce *T. rhodesiense* to its human host. In other words, he supposes that *T. rhodesiense* has become modified into the *T. gambiense* type in its endeavour to adapt itself to man. The difficulty about Kleine's view is to find an explanation for the virulent *rhodesiense* form of this human parasite. Kleine suggests that the greater virulence of *T. rhodesiense* can be explained by the unpreparedness of the human host in regions where a human trypanosome appears for the first time. This explanation is, however, not very convincing, and the alternative theory, namely, that *T. brucei* when it first obtains a footing in man will take time to settle down and adjust itself to its new host, is at least equally probable.

In the laboratory, long contact with one species of host often leads to the enhancement of the virulence of a trypanosome ; but there is a great difference between the natural method of cyclical passage from host to host through tsetse and the direct method normally in use in the laboratory.

It is tempting to suppose that a strain of *T. gambiense* hitherto dependent on *G. palpalis*, if introduced, in man, into an inhabited *morsitans* area, might appear in the hitherto clean population of that area in the guise of *T. rhodesiense*, the transformation being due to passage through an unfamiliar vector. But Kleine does not invoke this explanation ; in fact, his own investigations led him to conclude that there was no significant difference between *G. morsitans* and *G. palpalis* in this respect (Kleine and Fischer, 1913). The little experimental evidence hitherto accumulated about the effect exerted by the species of tsetse on the trypanosomes it carries has been acquired at Entebbe, where *T. brucei* and *T. rhodesiense*, both essentially dependent on game tsetse in nature, have been passed cyclically from host to host, by *G. palpalis* for several generations. In these experiments, which are still in progress, no alteration of virulence has yet been observed. As suggested in the Final Report of the League of Nations Commission, investigations along these lines are undoubtedly desirable (Duke, 1927). On the other hand, there is, as far as I know, no experimental support for the view that a trypanosome on virgin soil tends to develop increased virulence, as do the organisms of tuberculosis or syphilis. The numerous strains of *T. gambiense* studied at Entebbe must have been totally unfamiliar with the species of animals they met at that laboratory, yet they behaved in them in the normal traditional manner.

In short, there is very little certainty about this important subject. At the present moment the only way to distinguish *T. rhodesiense* from *T. brucei* is to use man as a test animal, and in our appreciation of the three important polymorphic trypanosomes we stand very much where we were twenty years ago. But, while knowledge is slowly accumulating about a number of subsidiary points, there are two important questions that can be settled by direct experiment without much further delay. It is known that *T. rhodesiense* can be introduced into game animals, and it is plain that this must happen in nature. We can now ascertain how the trypanosome behaves in these animals ; whether they are good hosts in the biological sense ; and whether after prolonged sojourn in their blood the trypanosome can still infect man. These are among the objects of experiments now to be described.

It is intended to carry out two lines of experiment. In one, the trypanosome will be examined from time to time in one and the same individual antelope. In the other, it will be passed cyclically by tsetse from antelope to antelope, remaining a few months in each animal.

The main object of this research is to find out whether, after a year or two in antelope, *T. rhodesiense* still retains its power of infecting man. To the administrator the answer to this question is of real importance, likewise to those interested in the preservation of the big game throughout Central and Southern Africa.

The conditions for carrying out this experiment at Entebbe are particularly favourable. The accommodation for the animals is excellent, and already a number of antelope are established in apparent comfort and contentment. An unlimited supply of *G. palpalis* pupae is at hand, and pupae of *G. morsitans* have hitherto been available in sufficient quantities from Tanganyika, so that transmission can be performed with either or with both species. There is, moreover, no danger of casual infection from wild fly.

The present paper describes the start of this investigation. Its successful completion depends upon the provision of sufficient time, continuance of the present adequate facilities and the avoidance of intercurrent disease and accident.

According to the literature available at this laboratory, the only other investigator to study the behaviour of *T. rhodesiense* in antelope is Dr. J. F. Corson, of the Tinde Laboratory, Tanganyika. Corson, in 1930-31, inoculated 4 dik-dik from a testse free area with strains of *T. rhodesiense* recently isolated from man. He reports that the animals appeared to suffer little in general health during a period of about a month. The object of his investigation was to determine whether the central nervous system was affected, and the animals were either chloroformed or treated with Bayer 21, 34, 69 and 33 days after infection. Posterior nuclear forms were found in the blood of the antelope (Corson, 1931).

II. HISTORY OF THE STRAINS OF *T. RHODESIENSE* USED IN THESE EXPERIMENTS

In April, 1932, Dr. Fairbairn, acting for Dr. Corson at the Tinde Laboratory, Tanganyika Territory, very kindly sent us a number of strains of *T. rhodesiense* recently isolated from natives by inoculation of their blood into white rats. Three of these strains were chosen for the infection of the antelope at Entebbe, the blood of each of 3 infected rats received from Tinde being inoculated into a separate monkey.

The first strain, Tinde 1, inoculated into monkey 949, came from native Nakunubwo, a female who had been ill for some 2 months. Her blood had been inoculated on 28.2.32 into a white rat (incubation period in the rat, 17-20 days); from this first rat a second was inoculated on 10.4.32, and from this second animal monkey 949 was inoculated on 18.4.32, at Entebbe.

The second strain, Tinde 2, came from Hamwingili, a male 30 years old, who had been ill for about 1 month. His blood was inoculated into a rat on 7.3.32 (incubation period, 7 days); from the rat a second was inoculated on 10.4.32, and from this animal monkey 950 was inoculated on 18.4.32, at Entebbe.

The third strain, Tinde 3, came from Tambuta, a male aged 30. First isolation in a white rat on 21.2.32, from which a second was inoculated on 4.4.32; monkey 923 was infected from this rat at Entebbe on 29.4.32.

These three strains, which have been examined in various ways at the Institute, do not differ from one another in their animal reactions and behaviour in the adhesion test. The first strain, known henceforth as Tinde 1, possesses a character which distinguishes it from the other two, a character not hitherto included among the recognised attributes of a strain of trypanosomes.

The type of gland infection produced in *Glossina* by Strain 1 differs from those produced by the other two strains. Strains 2 and 3 are characterised by very heavy gland infections, the whole gland being filled with a mass of flagellates. In a mature gland infection with Strain Tinde 1 the flagellates are always much less numerous, and are sometimes so scanty throughout the whole length of the glands and their ducts that very careful search is necessary to find them. At first no particular significance was attached to this phenomenon, but its constant occurrence, alike in flies of the third, fourth and fifth decades of the cycle, and in flies infected from antelope or from monkeys, is noteworthy, and suggests that this sparse type of gland infection is indeed a definite character of Strain 1.

III. SCHEMATIC REPRESENTATION OF THE HISTORIES OF THE INFECTIONS OF THESE ANTELOPE AND THE OTHER GAME ANIMALS DESCRIBED BELOW

In these schedules, all the passages are made by cyclical transmission through laboratory-bred tsetse. The date in brackets under an animal's experimental number is the date on which that animal was first bitten by an infected tsetse, i.e., the date of the animal's first infection with *T. rhodesiense.* The letter *m* means that the passage was effected by *G. morsitans*, *p* by *G. palpalis.* Pig *a* = wild pig of species *Potamochoerus choeropatomus* ; Pig *b* = wild pig of an undetermined species.

MONKEY 949

- p Monkey 924 (24.5.32)
- p Monkey 962 (4 6.32)
- p Reedbuck I (28 5.32)
- p Pig *a2* (30.5.32)
- p Pig *a* 'Horace' (2.6.32)

MONKEY 950

- p Monkey 963 (6.6.32)
- p Monkey 922 (6.6.32)
- p Ntalaganya I (2.6.32)
- p Pig *a* 'Horace' (2.6.32)
- p Pig *a2* (10.6.32)

MONKEY 923

- p Guinea-Pig T.T. (15.6.32)
- p Pig *b5* (24.6.32)
- p Bushbuck I (21.6.32)
- p Pig *a4* (19.6.32)

REEDBUCK I (Clean flies first fed 7.9.32)

- m Monkey 1015 (3.10.32)
- m Oribi I (5.10.32)
- m Bushbuck II (13.10.32)

NTALAGANYA I (Clean flies first fed 15.9.32)

- m Bushbuck III (30.10.32)
- m Pig *a2* (17.10.32)
- m Serval Cat (20.10.32)

BUSHBUCK I (Clean flies first fed 12.9.32)

- m Monkey 1022 (8.10.32)

IV. THE ANTELOPE

The collection of game animals destined to serve in this investigation includes the following species : reedbuck, bushbuck, oribi, duiker, situtunga, wild pig (2 species), serval cat, jackal and badger. Before it is employed, each animal's blood is inoculated into clean monkeys and stained thick films of its blood are examined microscopically. All these animals were captured in tsetse-free areas in the Protectorate.

The antelope house is a commodious building with washable walls and floor and with its own water supply. There are two main compartments, one subdivided into 6 pens suitable for animals under special treatment, and the other with pens along one wall and communicating with two fenced-in recreation enclosures. A wooden partition pierced with small openings affords a readily accessible refuge for the smaller species.

In these quarters, the animals seem quite contented with their lot and are in splendid condition. The distribution of the sexes has hitherto been too uneven to allow of breeding in captivity, but in a few months' time we shall possess adults of both sexes of situtunga, reedbuck and ntalaganya (forest duiker). A young forest duiker was conceived and prematurely born at the laboratory, but unfortunately the pregnancy was not remarked in time to protect the mother from molestation by the other animals.

The carnivora are, of course, kept separately, and it is hoped eventually to include in the examination jackals, hyaenas and the large cats about whose susceptibility to the polymorphic trypanosome little is yet known.

THE INFECTION OF THE ANTELOPE

REEDBUCK I. A young adult female; intractable and very shy, the only member of the company which shows no signs of becoming tame. Leapt an 8-foot fence in an attempt to escape, being caught in the open wire fence surrounding the laboratory grounds. Since this episode, the fence has been considerably heightened, and she has now given up attempts to scale it.

On 28.5.32, was fed upon by laboratory-bred *G. palpalis* metacyclically infective with *T. rhodesiense*, Strain Tinde 1, derived from monkey 949. Ten flies with infected glands fed on this reedbuck : 7 of these were at the 32nd day of the cycle, and 3 at the 31st day, when they fed on the animal. Trypanosomes were first found in stained thick films of the animal's blood on 8.6.32. At the date of writing, March 16th, 1933, this reedbuck appears to be in excellent health. Two months or so after its infection it seemed slightly less sleek and a little thinner than usual, but this was at the time of its attempts to escape, and it is impossible to determine whether the trypanosome infection was responsible for this very slight and temporary loss of condition.

Bushbuck I. A male, three-quarters grown, which has been at the laboratory since infancy. On 21 and 22.6.32, the animal was fed upon by flies cyclically infective with Strain Tinde 3 derived from monkey 923. Seven flies with gland infections fed upon the bushbuck, 5 being at the 39th day of the cycle and 2 at the 31st day.

The blood was negative to daily thick-film examination from 8.6.32 until 29.6.32, when polymorphic trypanosomes were found for the first time. Examinations made on July 2nd and 4th were positive, on the 3rd negative, on the 6th and 7th positive, on the 9th negative, and from the 9th to 13th positive; thenceforth examinations ceased. At the date of writing, this animal is apparently in perfect health and has at no time since its infection shown any signs of indisposition.

Ntalaganya I. (*Cephalopus caerulus melanorrheus*) Uganda blue forest duiker. An adult male.

On 2.6.32, was fed upon by flies cyclically infective with Strain Tinde 2 derived from monkey 950.

A stained thick blood film taken on 8.6.32 was negative: on the following day polymorphic trypanosomes were found in the duiker's blood.

The animal on 20.9.32 died of apparently uncomplicated trypanosomiasis. The duration of the disease was thus 110 days. The post-mortem examination was carried out by Mr. R. W. M. Mettam, the Veterinary Pathologist, Entebbe, whose report is as follows:—

'The carcase was that of a young male blue duiker which had been dead for about 4 hours. Rigor mortis was very marked.

'The animal was in very good condition. There were no lesions observable on the skin.

'The Blood was very tarry in colour, unusually viscid, and clotted rapidly. It stained intensely. The musculature and subcutaneous tissues presented no unusual features.

'The Spleen was somewhat contracted. On section its pulp appeared dry, but its colour was normal. The malpighian corpuscles were not prominent.

'All the Lymphatic Glands of the body were increased to about twice their normal size. On section they appeared swollen, and a copious milky exudation oozed from the exposed surfaces. No haemorrhages or pigmentation of the glands were noticed.

'Both Kidneys showed prominent streaky or dot-like haemorrhages throughout the cortex. The medulla appeared normal. Both organs were rather friable.

'The Liver was slightly swollen and dark mahogany in colour. The capsule showed no lesions. On section dark tarry blood oozed freely from the parenchyma which was swollen, very dark in colour, and distinctly friable. Lobulation was not at all distinct.

'The Rumen, Reticulum, and Omasum showed no abnormal features. They all contained soft, well masticated ingesta which appeared quite normal. The mucosae of the Abomasum was a deep rose pink in colour, very slightly swollen, and showed about 20 prominent ulcers, the majority of which were on the laminae. The ulcers were irregular in size and shape, and were comparatively shallow, being confined to the mucosa. They were all surrounded by a narrow red inflammatory zone.

'The Intestinal Tract contained very pultaceous faeces, but the mucosa was only hyperaemic in a few small areas, particularly along the Ileum. Nothing unusual was observed in any of the other abdominal viscera.

'In the Thorax the Lungs were a deep pink colour and free from any pneumonic lesions. In consistence they felt very doughy. On section a foamy pink oedematous fluid escaped especially on slightly squeezing the organs.

'About 6 pin-head bright red petechiae were observed scattered over the pericardium, while two similar haemorrhages were noted on the endocardium of the Left Ventricle. The valves and myocardium were normal.

'The Skeleton showed no unusual features. No fractures recent or healed, were found.

'The Nervous System showed nothing beyond stasis in the meningeal capillaries.'

This animal came from the forests of Chagwe. In its natural environment the ntalaganya in this part of Uganda will certainly never meet with tsetse. This little duiker lives in dense forest and its only contact with the outside world is through the medium of native hunters who attach considerable value to its skin.

The above three animals constitute the first generation of infected antelope, all of them being infected from monkeys carrying trypanosomes recently isolated from man. Infection of all three animals was brought about by laboratory-bred *G. palpalis*.

The second generation

ORIBI I. A young nearly adult female.

On 5.10.32, was fed upon by 2 laboratory-bred *G. morsitans* whose glands were infected with *T. rhodesiense*, Strain Tinde 1, acquired from Reedbuck I. *T. rhodesiense* was first seen in a stained film of the oribi's blood on 12.10.32. Up to the time of writing this animal has been in excellent condition.

BUSHBUCK II. A male, three-quarters grown, which arrived at the laboratory as an unweaned kid.

On 13.10.32, was fed upon by the 2 flies that infected Oribi I. These 2 flies were at the 28th day of the cycle when they bit Bushbuck II.

Daily examination of stained thick films of the animal's blood revealed nothing until 21.10.32, when polymorphic trypanosomes first appeared in the blood. Since its first arrival at the laboratory the animal has always enjoyed excellent health.

BUSHBUCK III. A three-quarters grown male, which first reached the Laboratory as an unweaned kid. On 30.10.32, was inoculated subcutaneously with a saline emulsion of the infected glands of a *G. morsitans* that had acquired its infection from Ntalaganya I.

T. rhodesiense was first found in a stained thick film of this bushbuck's blood on 9.11.32. Up to the date of writing the animal has been apparently in perfect health.

V. ACCOUNT OF THE TRANSMISSION EXPERIMENTS

(1) Transmission of *T. rhodesiense* from the monkeys 949, 950, 923, to the antelope of the first passage.

T. rhodesiense = Strain Tinde 1. Monkey 949.

eri- nt ber	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	Total		In gut only	In gut and salivary glands		
26	949	8	42	24	27	51	50	3	7	8, 34, 51, †34, †36, †36, †37, †37, †48, †49.	26, 28.4.32
27	949	7	40	21	28	49	48	0	6	†28, †31, †33, †33, †34, †48.	27, 29.4.32
29	949	5	39	29	22	51	45	...	...	...	29.4 & 1.5.32
30	949	5	28	22	15	37	44	...	...	...	30.4 & 2.5.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
April 26th to May 23rd	188	16	8·5	13	15	7·3

NOTE.—In all these transmissibility tables a † indicates a fly with infected salivary glands.

were irregular in size and shape, and were comparatively shallow, being confined to the mucosa. They were all surrounded by a narrow red inflammatory zone.

'The Intestinal Tract contained very pultaceous faeces, but the mucosa was only hyperaemic in a few small areas, particularly along the Ileum. Nothing unusual was observed in any of the other abdominal viscera.

'In the Thorax the Lungs were a deep pink colour and free from any pneumonic lesions. In consistence they felt very doughy. On section a foamy pink oedematous fluid escaped especially on slightly squeezing the organs.

'About 6 pin-head bright red petechiae were observed scattered over the pericardium, while two similar haemorrhages were noted on the endocardium of the Left Ventricle. The valves and myocardium were normal.

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'The Nervous System showed nothing beyond stasis in the meningeal capillaries.'

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The above three animals constitute the first generation of infected antelope, all of them being infected from monkeys carrying trypanosomes recently isolated from man. Infection of all three animals was brought about by laboratory-bred *G. palpalis*.

The second generation

Oribi I. A young nearly adult female.

On 5.10.32, was fed upon by 2 laboratory-bred *G. morsitans* whose glands were infected with *T. rhodesiense*, Strain Tinde 1, acquired from Reedbuck I. *T. rhodesiense* was first seen in a stained film of the oribi's blood on 12.10.32. Up to the time of writing this animal has been in excellent condition.

Bushbuck II. A male, three-quarters grown, which arrived at the laboratory as an unweaned kid.

On 13.10.32, was fed upon by the 2 flies that infected Oribi I. These 2 flies were at the 28th day of the cycle when they bit Bushbuck II.

Daily examination of stained thick films of the animal's blood revealed nothing until 21.10.32, when polymorphic trypanosomes first appeared in the blood. Since its first arrival at the laboratory the animal has always enjoyed excellent health.

Bushbuck III. A three-quarters grown male, which first reached the Laboratory as an unweaned kid. On 30.10.32, was inoculated subcutaneously with a saline emulsion of the infected glands of a *G. morsitans* that had acquired its infection from Ntalaganya I.

T. rhodesiense was first found in a stained thick film of this bushbuck's blood on 9.11.32. Up to the date of writing the animal has been apparently in perfect health.

V. ACCOUNT OF THE TRANSMISSION EXPERIMENTS

(1) Transmission of *T. rhodesiense* from the monkeys 949, 950, 923, to the antelope of the first passage.

T. rhodesiense = Strain Tinde 1. Monkey 949.

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	Total		In gut only	In gut and salivary glands		
626	949	8	42	24	27	51	50	3	7	8, 34, 51, †34, †36, †36, †37, †37, †48, †49.	26, 28.4.32
627	949	7	40	21	28	49	48	0	6	†28, †31, †33, †33, †34, †48.	27, 29.4.32
629	949	5	39	29	22	51	45	...	...	...	29.4 & 1.5.32
630	949	5	28	22	15	37	44	...	...	...	30.4 & 2.5.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
April 26th to May 23rd	188	16	8·5	13	15	7·3

NOTE.—In all these transmissibility tables a † indicates a fly with infected salivary glands.

T. rhodesiense = Strain Tinde 2. Monkey 950.

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	Total		In gut only	In gut and salivary glands		
628	950	6	33	25	26	51	48	2	0	6, 7.	28, 30.4.32
631	950	6	43	24	29	53	47	...	...	...	1, 3.5.32
632	950	9	45	27	22	49	45	1	7	29, †24, †31, †31, †40, †41, †41, †42	2, 4.5.32
636	950	6	40	17	29	46	40	0	1	†31	7, 9.5.32
637	950	5	25	26	20	46	40	9	1	5, 8, 10, 10, 12, 13, 15, 15, 23, †32	7, 9.5.32
641	950	6	32	29	15	44	38	3	2	6, 9, 14, †30, †32	10, 12.5.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
April 28th to May 12th, 1932 ...	289	26	9·0	11	12	8·2

T. rhodesiense = Strain Tinde 3. Monkey 923.

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	Total		In gut only	In gut and salivary glands		
646	923	5	42	34	16	50	41	3	8	10, 41, 41, †26, †31, †31, †41, †41, †41, †41, †41	13, 15.5.32
654	923	16	39	26	24	50	37	0	1	†28	17, 19, 21.5.32
655	923	8	36	27	24	51	29	0	1	†39	17.5.32
657	923	13	45	32	31	63	33	1	2	13, †33, †33	16, 18.3.32
658	923	7	34	21	29	50	40	3	0	9, 9, 9	19.5.32
662	923	4	31	26	21	47	37	2	2	20, 20, †37, †37	21.5.32
674	923	5	37	30	20	50	33	3	0	7, 9, 11	28, 30.5.32
675	923	5	17	26	26	52	33	1	1	9, †33	...
685	923	6	18	17	10	27	25	2	0	6, 11	2, 3, 4.6.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissiblity index
				With glands infected	Total	
May 13th to June 5th, 1932 ...	440	30	6·8	15	17	6

(2) Transmission of *T. rhodesiense* from the antelope of the first to those of the second passage.

Reedbuck I

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feed
				Males	Females	Total		In gut only	In gut and salivary glands		
914 (m)	Reed-buck	7	22	20	24	44	39	1	1	26, †21	6, 7.9.32
915 (m)	”	7	26	20	23	43	39	7	0	15, 15, 22, 31, 39, 39, 39	...
916 (m)	”	8	25	22	30	52	38	6	1	19, 20, 21, 24, 38, 38, †38	7.9.32
917 (m)	”	6	31	25	24	49	38	3	1	14, 14, 16, †38	...
925 (m)	”	5	20	21	19	40	33	...	...	...	9, 11.9.32
926 (m)	”	10	30	15	27	42	33	4	0	15, 22, 33, 33	...
927 (m)	”	6	21	22	22	44	33	2	0	22, 27, 0	...
928 (m)	”	7	20	24	23	47	33	...	...	...	...
933 (m)	”	6	12	14	17	31	34	2	0	14, 20	11, 13.9.32
934 (m)	”	7	18	19	17	36	36	2	0	13, 16	...
943 (m)	”	7	18	22	22	44	34	8	1	20, 20, 24, 25, 30, 34, 34, 34, †34	13.9.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Trans-missibility index
				With glands infected	Total	
September 6th to 13th, 1932 ...	472	39	8·2	4	26	1·2

Bushbuck I

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	Total		In gut only	In gut and salivary glands		
937 (m)	Bushbuck	6	23	19	24	43	34	1	0	34	12.9.32
938 (m)	”	7	26	22	21	43	35	...	...	...	...
939 (m)	”	7	26	18	27	45	35	0	1	†33	...
940 (m)	”	5	24	23	20	43	34	1	1	34, †31	...
950 (m)	”	6	29	33	22	55	36	...	...	...	14.9.32
951 (m)	”	6	18	19	25	44	36	...	...	...	...
952 (m)	”	7	27	22	23	45	36	1	0	19	...
953 (m)	”	7	25	25	20	45	36	...	...	...	...
963 (m)	”	6	21	27	15	42	37	5	0	37	16, 17.9.32
964 (m)	”	7	23	30	12	42	37	4	0	19, 37, 37, 37	...
967 (m)	”	8	21	27	14	41	36	4	0	16, 36, 36, 36	17.9.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
September 12th to 17th, 1932 ...	488	18	3·6	2	15	0·4

Ntalaganya I

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feed
				Males	Females	Total		In gut only	In gut and salivary glands		
954 (m)	Ntalaganya	5	19	23	23	46	38	4	0	5, 7, 10, 15	14.9.32
955 (m)	„	6	20	22	18	40	38	2	0	10, 12	...
958 (m)	„	7	17	20	18	38	46	0	1	†46	15.9.32
959 (m)	„	7	28	31	21	52	46	6	1	22, 24, 38, 42, 43, 46, †46	...
970 (m)	„	9	12	22	10	32	43	7	1	12, 12, 14, 15, 16, 24, 34, †43	18, 19.9.32
971 (m)	„	7	17	24	13	37	43	1	0	34	...

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
September 14th to 19th, 1932 ...	245	23	9·3	3	9	3·1

VI. *T. RHODESIENSE* IN GAME ANIMALS OTHER THAN ANTELOPE

Wild Pig—'Horace' (*Potamochoerus choeropatomus*). This animal has already been used in an investigation of the animal reservoir of *T. gambiense* (Duke, 1931). Its previous history is briefly as follows :—

Preliminary testing by sub-inoculation of the pig's blood into clean monkeys and also by feeding upon it clean laboratory-bred flies proved the animal free from infection.

The pig was then infected on 13.7.29 with *T. gambiense* by feeding upon it flies infective with *T. gambiense*, Strain XXXIII. Clean laboratory-bred *G. palpalis* were then fed upon the pig as is shown in the following summary :—

Test	Date	Total flies dissected	Total infected flies	Per cent. of infected flies	Number of infected flies alive at 25th day		Trans-missibility index
					With glands infected	Total	
1	25.7 to 22.8.29	518	2	0·3	1	1	0·3
2	19.9 to 6.10.29	417	1	0·2	0	0	0
3	19.5 to 24·6·30	408	1	0·2	0	1	0
4	21.8 to 17.9.30	406	0	0	0	0	0

On 3.6.30, two, and on 10.10.30, one, sub-inoculations of this pig's blood were made into clean monkeys, but the animals never became infected.

Between October, 1930, and November, 1931, the animal was left in peace and attained its full growth. Daily examination of stained thick blood smears was then recommenced and continued until the end of March, 1932. No trypanosomes were seen in these films.

On 2.6.32, the pig was fed upon by the *G. palpalis* of experiments 626 and 627 which were carrying *T. rhodesiense* Strain Tinde 1 (cf. above). Three flies with infected glands fed on the pig.

On 8.6.32, *G. palpalis* of experiment 632, infected from monkey 590 (see above), were fed on the pig; four of the flies that bit the animal had heavy gland infections. Daily examination of stained thick films of the pig's blood failed to reveal trypanosomes for the ensuing two months, after which examination was stopped. Three hundred and fifty-seven clean laboratory-bred *G. palpalis* were fed on the pig between 23.6.32 and 3.7.32; none became infected.

Finally, on 27.10.32, 4 c.c. of the animal's blood were inoculated into a clean monkey and no infection resulted.

WILD PIG NO. 2. A quarter-grown female *P. choeropatomus*. Preliminary examination of stained thick films over a considerable period, and also a sub-inoculation into a clean monkey, failed to reveal any signs of trypanosome infection in this young animal, which was caught in a fly-free area.

On 13.11.31, it was bitten by *G. palpalis* carrying a strain of *T. rhodesiense* sent to us some months previously by Dr. Corson from Tanganyika. This strain had been isolated from man on 2.7.31 by inoculation into a white rat. Daily examination of stained thick blood films of the pig from 16 to 21.10.31 were negative; on 23.11.31 the blood contained trypanosomes, also on the 24th, the 25th and on 1-2.12.31. From 17.12.31 until 14.6.32, daily blood slides were negative. On 21.1.32 and 4.4.32, the pig's blood failed to infect clean monkeys.

On 30.5.32, Pig No. 2 was bitten by 10 *G. palpalis* with gland infected with *T. rhodesiense* from monkey 949 (cf. above), and on 10.6.32, by 6 more flies infected with the same strain—16 infective flies in all.

On 15.6.32, the pig's blood contained polymorphic trypanosomes.

On 17.10.32, three *G. morsitans* with infected glands, carrying Strain Tinde 2 from the Ntalaganya, fed on Pig 2.

On 31.10.32, a clean monkey was inoculated with the pig's blood; trypanosomes were first seen in the monkey's blood on 23.11.32, and it died on the following day. The pig died on 23.11.32, and a second clean monkey, inoculated with the pig's blood on 23.11.32, remained negative to trypanosomes and died on 16.1.33.

Early in October, 1932, a swelling was noticed in the region of the angle of the jaw of this pig. The swelling proved to be due to the tubercle bacillus. Mr. Mettam performed the post-mortem on the pig and found a widely distributed and intense tubercular invasion, the bacillus being subsequently proved by Mr. Carmichael, Assistant Veterinary Pathologist, to be of the bovine type. The monkey inoculated with this pig's blood on 23.11.32 also developed acute tuberculosis, including an abscess containing tubercle bacilli at the site of the subcutaneous inoculation of the pig's blood, and miliary tubercles all through the lungs, the mesentery and the spleen.

Pig No. 2 thus underwent three separate infections with *T. rhodesiense*. The first infection resulted in the appearance of trypanosomes in the peripheral blood for a week or so, after which they were seen no more. The condition of the pig during this first infection showed nothing of note. The second infection, also produced by cyclically infected tsetse, occurred some 5½ months after the first. For some time afterwards the pig showed no deterioration in health, and then the swelling of the glands commenced. The third exposure to infection took place some 4 months later. The tubercular infection was perhaps contracted from the milk which the animal consumed while still young, its diet for the last 9 months of its life being entirely vegetarian.

During the last few days another wild pig of about the same age has developed a similar glandular enlargement strongly suggestive of the same cause. The two animals were in adjoining pens, though not in direct contact, and the second animal has never been infected with trypanosomes.

The following experiments with clean laboratory-bred *G. palpalis* were performed on Pig No. 2. In December, 1931, 243 flies were fed on the animal: 2 developed trypanosome infections, but both flies died too early for a gland invasion to take place.

In March, 1932, 410 clean flies were fed upon this pig and 2 became infected, both dying on the 16th day of the cycle. In late June and early July, 1932, 287 flies failed to pick up any trypanosome from this pig. The animal died of acute tuberculosis. The fact that a clean pig of the same species a few months older has also developed the disease suggests that the trypanosome infection need not be invoked as a predisposing cause of the tuberculosis. There is no evidence that Pig No. 2 suffered any inconvenience from its trypanosome infection. As with Pig No. 1, it was found very difficult to infect clean *G. palpalis* fed upon the animal even at the time when trypanosomes were actually present in its blood: in this respect, there is a great contrast between the wild pig and the antelope described above.

WILD PIG No. 4. A quarter-grown wild pig of the same species as Nos. 1 and 2 and also caught in a tsetse-free area. Preliminary examination, including blood examination over several months and inoculation into susceptible animals, revealed no signs of trypanosomes.

On 19.6.32, was fed on by 7 gland-infected *G. palpalis* carrying *T. rhodesiense* Strain Tinde 3. The animal's blood was negative to daily examination from 18.6.32 until the end of October.

On 29.10.32, a sub-inoculation of the pig's blood was made into a monkey and no infection resulted; likewise on 11.1.33, on 24.1.33 and on 31.1.33, always without producing infection. In spite of a heavy exposure to *T. rhodesiense*, the animal apparently never became infected, although the flies that bit it had infected other clean animals previous to biting the pig.

Post-mortem report by Mr. R. W. M. Mettam is as follows:—

'The cause of death was impaction of the small intestine by masses of round worm, i.e., *Ascaris lumbricoides*, which were present in all phases of development. Altogether over 200 specimens were recovered from small intestine, caecum and colon. There was also a mild bilateral broncho-pneumonia.

'All other organs were perfectly healthy.'

WILD PIG NO. 5. A dark coloured animal with fine white whiskers; species undetermined. Caught in the Eastern Province, in a fly-free area. Blood negative to preliminary examination.

On 24.6.32, fed upon by 3 *G. palpalis* whose glands were infected with *T. rhodesiense* Strain Tinde 3,

On 28 and 29.6.32, polymorphic trypanosomes were seen in the animal's blood; on 18.1.33, 4 c.c. of its blood were inoculated into each of 2 clean monkeys; neither monkey became infected. The pig died on 18.1.33, being chloroformed when *in extremis*. From October 5th to 17th, 1932, 272 clean laboratory-bred *G. palpalis* fed on this animal but none of the flies became infected. The post-mortem was performed by Mr. Mettam, whose report reads as follows :—

'The post-mortem showed intense jaundice of all serosa and mucosa. The liver however appeared perfectly normal.

'There was about 200 c.c. sanguineous fluid in the peritoneal cavity and a considerable number of soft fibrinous adhesions between the viscera. No cause could be found for this condition.

'The animal was in good condition and showed no sign of tuberculosis.

'The heart and kidneys appeared normal but the spleen was distinctly swollen and the pulp tumescent.

'There was a mild haemorrhagic gastro-enteritis.

'The cause of death is apparently very obscure. The lesions affecting the intestinal canal and spleen might well be due to trypanosomiasis but it is impossible to explain the acute peritonitis. Possibly it was due to an injury but none could be found.'

'Here again there is no evidence that the trypanosome contributed to the death of the animal. Apparently the infection was of a transitory nature, and here again the failure of the pig's blood to infect clean *G. palpalis* is worthy of note.

SERVAL CAT. A young adult animal in excellent condition, which had been kept for some months in captivity.

On 20.10.32, was fed upon by 2 laboratory-bred *G. morsitans* whose glands were infected with *T. rhodesiense* Strain Tinde 2. The flies derived their infection from Ntalaganya No. 1. Trypanosomes appeared in the cat's blood on 30.10.32, and the animal died with keratitis and rapid wasting on 4.12.32. From November 14th to 26th, clean laboratory-bred *G. palpalis* were fed on the cat.

The results of these experiments were as follows :—

Serval Cat = 950 Strain : *T. rhodesiense*

Experiment number	Infected animal	Day of experiment on which dissection began	Number of flies alive on 25th day	Number dissected during the experiment			Duration of experiment in days	Number of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	Total		In gut only	In gut and salivary glands		
1096	Cat	4	40	26	31	57	33	1	0	12	14.11.32
1101	„	6	20	23	19	42	31	2	0	7, 7	16.11.32
1102	„	5	10	21	12	33	28	1	0	5	17.11.32
1103	„	7	35	22	22	44	28	...	...	...	...
1104	„	7	31	39	10	49	29	...	...	...	18.11.32
1105	„	6	28	20	17	37	28	...	...	...	19.11.32
1113	„	9	40	21	34	55	35	1	1	21, †34	24, 26.11.32
1117	„	8	36	24	34	58	32	2	0	12, 21	26.11.32

Summary

Date	Total flies dissected	Total infected flies	Percentage of infected flies	Number of infected flies alive at 25th day		Transmissibility index
				With glands infected	Total	
November 16th to 26th, 1932 ...	375	8	2·1	1	1	21

The susceptibility of the serval cat to *T. rhodesiense* possesses some practical interest, as it and its congeners are often found in game country. It is hoped in the future to be able to test this trypanosome in the big felines whose distribution extends through all the game tsetse belts of Africa.

VII. SUMMARY

1. A reedbuck, an ntalaganya (*Cephalopus caerulus melanorrheus*), 3 bushbuck, 1 oribi, 3 wild pigs (*Potamochoerus choeropatomus*) and 1 of an undetermined species and a serval cat have all been infected with strains of *T. rhodesiense* recently isolated from man.

2. Infection in every instance was caused by the bite of cyclically infected laboratory-bred tsetse, either *G. palpalis* or *G. morsitans*.

3. The reedbuck and bushbuck and oribi showed no noteworthy symptoms ascribable to the trypanosome infection. The ntalaganya died of uncomplicated trypanosomiasis in 110 days.

4. Clean laboratory-bred flies were infected from the reedbuck 107 days after the original infection of the animal; from the bushbuck 108 days after infection; and from the ntalaganya 87 days.

5. The wild pig (*Potamochoerus choeropatomus*) is susceptible to infection with *T. rhodesiense* but appears to be a very unsuitable host for the trypanosome. It is difficult to infect clean laboratory-bred *G. palpalis* from an infected pig. Another and undetermined species of wild pig behaved in the same manner.

6. A serval cat proved very susceptible to *T. rhodesiense* and died of the disease after 45 days. Clean laboratory-bred tsetse were readily infected from the cat's blood.

This investigation is still in progress and further reports will be issued as indications arise.

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THE TAXIES OF INFECTIVE HOOKWORM LARVAE

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In 1924, Professor Fülleborn wrote on taxies of infective strongyloides and hookworm larvae; in 1930, I dealt with the behaviour of hookworm larvae, the paper being in effect and deliberately a mere suggestion of reasons for hesitating to accept certain of my friend's conclusions. Professor Fülleborn having again (1932) taken up the matter, it falls to me to justify my rather ambiguous position by exact reasoning.

I wish first to remove some misconceptions. In his second paper, Fülleborn has made evident his belief that in mine I had questioned the validity of observations made by himself and others. I know of no observations of his which I have ever doubted. Indeed, I give place to no one in admiration for their accuracy and for the generous manner in which they have been described and pictured for the benefit of other workers. But unfortunately there is disagreement between us as to the conclusions which can legitimately be drawn from the facts. Again, it is clear that while we have used the same terms we have not always done so with exactly the same meanings. In what follows, then, it is very necessary that I should make quite clear both my own usage and the reasons for which I have been driven to certain conclusions.

Fülleborn (1924) has quoted Przibram's definition of a taxy as the movement of an animal in a definite direction under the influence of a directive stimulus. That is to say, the word tropism or taxy must express a vital act. Here is a trite illustration. To continue its species the seeds of an apple must of necessity reach the soil, but when in the ordinary course of events an apple falls to the ground, its actual motion through the air is due to purely physical forces; it is no vital act and is no tropism, although it subserves essential biological ends. From this standpoint, no movement or behaviour of the larvae of hookworms or of other nematodes can properly be attributed to the action of a taxy if it is capable of reasonable and simpler explanation on mere physical and mechanical grounds. Another point needs emphasis in connection with hookworm larvae. They have one kind of progressive movement only. Accordingly, in considering the presence of any taxy only one question arises : Does the stimulus in question give direction to the ordinary larval movements or does it not ? That it gives rise to no new kind of movement does not negative the presence of a taxy.

I. HYDROTAXY

I have stated (1930) that since hookworm larvae are obligatorily aquatic it is unreasonable to suggest that an animal already surrounded by water should be attracted to water elsewhere, however large any second body of water may be. Fülleborn replies that infective larvae do not in fact live of necessity in water, moist earth being their ordinary habitat. Here is misunderstanding. When I wrote ' water ' I meant quite exactly H_2O in the liquid form, and in that connection I quoted from a paper by Florence Payne (1923), in which she describes her valuable observations on these larvae in their natural habitat, the water-film on and between soil particles. A microscopic quantity of liquid H_2O suffices to enclose a microscopic animal, and moist earth abundantly fulfils these conditions. Three examples may be quoted to clarify this point. Looss (1911, p. 398, quoting Looss, 1903, p. 335) sums up the evidence that even partial drying of a larva, if carried beyond a certain point, causes its death ; complete drying is inevitably fatal. Moreover, as he notes, Leichtenstern (1887, p. 669), put aside a faecal mass for 271 days, by which time it had become so dry that it could be scraped with a knife, yet it then contained a few active infective larvae. I have myself (1932) produced more exactly measured evidence on this matter. A culture of one part of faeces and four parts of earth may be so crumbly and dry-looking as to seem a very improbable habitat for aquatic life. Yet one such culture had 8 per cent. of water in it and contained many very active infective larvae. The evidence, then, is that infective hookworm larvae die inevitably if dried, but, being microscopic animals, the amount of liquid H_2O which they need for life is microscopic in quantity. Since, then, they do in fact live in water, a suggestion that they have a taxy urging them to proceed to another body of water is unlikely to be correct, and when, as is explained below, it is in fact unsupported by evidence, it is unacceptable.

Two matters may be brought forward against this conclusion. One is that larvae in the substance of agar are not in liquid H_2O, but yet they live. Regarding this, it must be noted that it is drying which is fatal to larvae and that the agar plates used by Fülleborn contained 97 to 98·5 per cent. of water. Quite obviously, larvae which lie within such agar and not in the water of condensation which lies on its surface are in jellied, not in liquid, H_2O; and, equally evidently, prepared agar is not a medium which they meet in nature. It rests with readers to make up their minds whether this consideration annuls the conclusion that hookworm larvae are obligatorily aquatic.

The second matter is one specifically mentioned by Fülleborn, Augustine (1922) taking the same view. Regarding the facts, there is no question. When tufts of larvae extrude from cultures they disappear when the air dries, to reappear when it is moistened. Fülleborn believes this to be the result of a hydrotaxy; I hold it to have a purely physical causation, to be in effect a duplication of what may be watched on a slide. That indeed was my reason for deliberate mention of the simple observation which is here detailed again; one in which, moreover, there seems to be control of single factors. When a spread-out drop of larva-containing water has been standing on a slide for a short time, most, probably all, active larvae will be found at its margins. As the drop lessens in area by evaporation, all, or perhaps only nearly all, larvae will still be within it, but they will be concentrated within the smaller area. They have been swept inwards by its contracting margin, held together as it is by its surface tension which the larvae have not been able to overcome. If the area is increased by the addition of water, the larvae shortly re-establish themselves at its margin. These are matters of readily verifiable observation. Equally a matter of verifiable observation is the behaviour of larval tufts on cultures which Fülleborn re-states in noting that one can in fact see the tufts of strongyloides larvae disappear from the gauze of a culture funnel when the air dries, to reappear when the air is sufficiently moist (1932, p. 163), and which he describes in greater detail when advising that, to obtain excellent tufts, the culture gauze should be sprayed with water while still in the warm chamber; the apparatus should then be removed to a cool place so that the air in it falls below the dewpoint (water, that is to say, will be deposited on it), and tufts will thereafter shortly appear (p. 177). But it will be noted that these are just the phenomena which, as observation of a drop of water on a slide shows, are produced by purely physical causes; for, when water-vapour condenses in the liquid form on a culture, larvae will invade the dewdrop just as they do added water on a slide, will shortly reach its margin, and, if in sufficient numbers and having sufficient vigour, may be able to push it out into tufts which are held together by the surface tension of the film of water which encloses them. They will stretch this surface to the limit of their power. But as the water again evaporates its surface tension pushes inwards with increasing force, and the larvae, no longer having sufficient power to 'tent' it, are forced back resisting into the apparatus, and the tuft disappears. That is to say, as stated above, the alterations are those which the physical forces known to be at work must produce; they do not constitute evidence for hydrotaxy. In other words, no evidence for hydrotaxy has been obtained, and in its absence the serious consideration of this taxy as an explanation of larval behaviour was, and still is, held by me to be out of place.

II. RHEOTAXY

Fülleborn writes (1932) that I am not entitled to offer the objection that the arrangements of his investigation were ill-adapted to show rheotaxy, the objection having been made on the grounds that, in using these arrangements, one could see under the microscope only those larvae which remained in the microscopic field—only those, in fact, which were swimming just as fast as the stream flowed (Lane, 1930). He adds that, if I had repeated his experiments with sufficient care, I would have seen larvae swimming with the stream. I think the context makes it clear that I was describing my own experiments. In them the strength of the stream was such that only vigorously swimming larvae were kept in view, and that as soon as they ceased to do so they were carried out of sight " in a flash." Nevertheless, I seem to have erred, if I did err, in the best of company, since Fülleborn wrote (1924, p. 162) that if one hits on the right velocity of stream the larvae remain in the field of vision, their movements approximately balancing that of the current. The matter, however, is of minor importance, for I have shown (1930) that even motionless larvae turn and head upstream, a change which can be due to nothing but the action of physical forces, and one which is exactly what would be expected from consideration of the shape, and so of the presumed balance, of the larvae themselves. Accordingly, to call in as an explanation of the observed facts the unevidenced influence of a rheotaxy would be against the principles which have been suggested above as being the only ones which have a satisfactory foundation.

III. CHEMICAL ACTIVATION

Fülleborn has suggested (1932) that the increased activity among larvae which I have observed on addition of serum to the fluid in which they lie is due to this substance causing them to adhere to one another or to other matter, thus restricting their movements ; in fact, that it is due to the bringing into play of what I have suggested below to be a negative thigmotaxy. Nothing of the kind occurred in the weak solutions which I used ; there was no clumping, no adhesion, no restriction of movement. My own suggestion (1932, pp. 65, 66) is that the question of the possible absorption of nutrient matter through the sheath cannot summarily be rejected. For one must suppose that as soon as the larval cuticle has separated from its body to form a sheath, that sheath is dead, irrespective of whether the larva within is dead or alive. Now Leichtenstern showed (1887, p. 671) that a larva could be kept alive and apparently unharmed in gastric juice for at least two days, but that as soon as it died it was rapidly digested within its intact sheath. It must then be concluded that pepsin and hydrochloric acid penetrate the intact sheath, so that to rule out arbitrarily the possibility that other substances can do the same is not reasonable. Hirst (1932) cites an extraordinary experience in which heavy hookworm infection seems to have been caused by digging in land which had been trenched at least three years earlier In discussing it, he points out that there is no present evidence that infective hookworm larvae do in fact obtain nourishment through their sheaths ; and it is, of course, not questioned here that their reserve granules form a notable source of nourishment for larvae waiting for the chance to infect. But to say in face of the evidence cited that these granules can form their only possible source of nourishment would be inconsequential; while if they can so use outside matter the very varying life spans of different batches of infective larvae may therein find an explanation, and at the same time a means towards lessening the duration of soil infectivity may possibly be supplied to us.

IV. GAS TAXIES

I confess to continued scepticism regarding the validity of the conclusions drawn by Fülleborn (1924) on taxies called out by oxygen, hydrogen and carbon dioxide. I have made no experiments of my own, so that my personal opinion may perhaps be judged to carry no great weight.

V. THIGMOTAXY

Fülleborn (1924) describes thigmotaxy of zoology as a directing impulse produced by contact with stable objects. It is in this exact sense that I have used the term. To that definition I strictly adhere. I reject as misleading Pintner's modification that for the calling out of thigmotaxy the stable body should have holes in it. In this instance, Professor Fülleborn and I have used the same word with different meanings.

Positive Thigmotaxy.

Concerning positive thigmotaxy, the question to be answered is this: Does contact of the anterior face of a hookworm larva with an obstacle direct the ordinary movements of the larva in such a way that it turns towards the obstacle? I have put forward two considerations as giving reason for concluding that it does. The first is that larvae set themselves roughly at right-angles to any obstacle which they encounter; the second is that, after larvae have been in an examination box (Lane, 1930, p. 414) for a short time, the place where nearly all of them will be found is not just anywhere, but precisely at its edges and angles. In practice it is here that, led by experience, one first looks for them either in a box or in a drop of water.

The tendency of larvae to set themselves roughly at right-angles to the surface against which they push is a matter of observation which can readily be tested in the examination box. Fülleborn (1932) cannot agree that this attitude can be taken up by larvae as the result of a directing influence, since a larva touches an object with its anterior face at a very tiny point only, almost a mathematical one. But there can hardly be a directing of its movements unless a larva is actually in movement; and, when it is so, its anterior end is not held still, but partakes in its general undulant motion. Moreover, its anterior face carries six cephalic papillae which, as Fülleborn himself notes, are presumably six sense organs; and as such they are, inferentially, fully adequate to give to larval movements a directional impulse according as the waving anterior face brings one or other into contact with any obstruction. The existence, then, of a directional impulse which sets a larva at right-angles to an opposing surface is primarily a matter of observation readily carried out, while reasoning suggests the anatomical and physiological means by which thigmotaxic stimulus can produce taxic movements. The second point is that in outstanding numbers larvae lie close to obstacles, just indeed where they would lie if influenced by positive thigmotaxy; for, as Fülleborn has noted (1924, p. 145), opposition to their movements is apt to activate these until they become almost convulsive. Thus is explicable their persistent presence at places where opposition is experienced—it has called out an increase of thigmotaxic movement.

Certain objections now fall to be considered. The first is that larvae are not in fact attracted to a body full of holes such as blotting paper. As pointed out above, the writer does not accept Pintner's views, and for these reasons. Positive thigmotaxy is viewed as taxic movements brought into being when the larval anterior face touches an object (*θίγμα*, touch), a meaning in accordance with its natural derivation and with the definition for zoology quoted by Fülleborn. When the restless anterior face of a larva has inevitably slipped off a partition between holes or clefts, such as is formed by a fibre of blotting paper, it slips into vacancy, it no longer touches anything, and at once ceases to be subjected to thigmotaxy, for naturally there cannot be a touch taxy in action when there is nothing to touch. But its ordinary movements inevitably carry it on right through the blotting paper or like substance in its search, if one may figuratively put it so, for some solid resistance to push against. There is next the question of the side-slipping of larvae and their collection in angles and bays of the margin of the fluid which contains them. The term 'side-slip' I have used as a convenient one for a transference which appears lateral to the observer. Fülleborn used it in the same sense (1932, p.164, line 1). As he notes, it implies dorso-ventral undulation of the larva (cf. Lane, 1932, p. 34). But these movements look symmetrical; they have no suggestion that they will drive the larva either ventrad or dorsad. Rather, other things being equal, will the direction of the slipping depend on the fortuitous average deviation from the vertical which the larva happens to possess at the moment of slip, together with the relationship between two points on the obstacle. The first point is a real one, namely that at which the larval head touches it; the second is imaginary, namely, that at which the line of force resulting from the larval movements intersects the face of the obstacle. At all events, these repeated side-slips do in fact tend to carry larvae, purely mechanically, into any angle or recess in the opposing object. That larvae do so collect is not in dispute; that their collection can be observed to occur in conditions which can reasonably be explained on purely physical grounds, provided positive thigmotaxy in its strict and accurate sense is postulated, seems to me equally obvious.

NEGATIVE THIGMOTAXY.

It is here suggested that under this heading it may be found reasonable to place certain larval reactions. Among them is that simultaneous swaying of close-set, parallel-lying larvae which Fülleborn has so delightfully photographed (1932, fig. 6). The first published appreciation of these synchronised larval movements seems to have been that by F. Smith (1905), when writing of the larval tufts or petals described by van Durme three years earlier. His words are: 'By narrowly watching the surface of the faeces you may see these marvellous collections of embryos form. One embryo rears up and waves itself in the air; another climbs up the first one, and perhaps waves about from the head of its supporter; then a third embryo will swarm up number one, and so on until a petal is formed.

All the members of a petal move in unison, and produce the curiously deceptive appearance of a single worm.' * Put into other words, the movements of a larval tuft are made up of movements of individual larvae so synchronised that those parts of the various individuals in a tuft, which lie at the same distance from the tuft's apex, move in the same direction, at the same time, and to the same degree, irrespective of the distance of this particular point from the head or tail of any individual larva in the tuft. At the same time, all larvae must have their dorso-ventral planes parallel, since it is only in this plane that movement occurs in nematodes. Exactly the same may be noted on the slide, as Fülleborn's cited figure shows. This striking phenomenon is, I suggest, reasonably explained by postulating in larvae a negative thigmotaxy to a touch on dorsal or ventral aspect—a retraction from contact. With larvae closely packed, such retraction of one larva will bring its opposite surface into contact with a second, which, too, will shrink away at the spot touched ; and in this way there may be produced, when there is close packing and parallelism of alignment and of dorso-ventral planes, that timed rhythmical swaying of the whole group which is here under consideration. Convulsive writhing movements, for example those described by Fülleborn (1932, p. 170) as occurring when a fibre of glass wool is laid across a larva, are reasonably explicable as non-rhythmic illustrations of the same negative thigmotaxy.

As Fülleborn notes in the legend to his fig. 6 (1932), the failure of larvae there pictured to reach the visible edge of the drop is due to no negative thigmotaxy, to no instinct to avoid butting their heads against the impenetrable, but simply to the shallowness of the drop. The larvae have pushed forward as far as they can go, but are held back from the visible edge of the drop because there is no room there for their heads. The space between the slide on one side, and the surface of the drop held together by its surface tension on the other, is too shallow for their entry; but the positive thigmotaxy called into being by the double contact, keeps them there. The same holds in his fig. 5 for the free edge of the drop, but not for the vaselined sides where the water is deeper. It holds too for his cinema strip (fig. 4), where the only spot at which the larva can reach the optical edge is where an artefact deepens the flat drop.

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* Italics not in original.

HYGIENIC IMPLICATIONS.

A positive taxy to touch has, then, a definite directive effect, apart from the fact that touch on the larval face accentuates the normal larval movements. It subserves the act of infection in some such way as this. When the anterior larval face touches the skin, larval movements are accentuated and directed more or less at right-angles to the epidermal surface. If the surface should here be smooth and resistant, side-slip is likely to carry the larva hence, but as soon as some microscopic concavity in the skin is reached there will be less roving, and if the base of the depression is weak there will be penetration. When this occurs, negative thigmotaxy, with its shrinking from touch on larval venter or dorsum, also stimulates movement; and progress is continued until a capillary vessel, blood or lymphatic, is reached, and the larval movements force it along the line of least resistance so that it boards the particular escalator, blood or lymphatic, which it has chanced to strike.

VI. THERMOTAXY

In my former paper, I sharply differentiated activation of larvae by heat from thermotaxy, first described by Khalil. Of the reality of the well-substantiated but fleeting activation of larvae by an increase in the surrounding temperature there will be no dispute. Regarding a directive stimulus by temperature, after discussion of the facts I wrote thus (1930): 'This consideration implies that, could there be produced mechanical larvae provided with quite erratic movements which increased in energy as the temperature rose to a certain point, and then ceased abruptly as it passed that point, there would, it seems, be produced just such a distribution and behaviour as that described by Fülleborn.' This sentence which summed up my attitude has not been mentioned by Fülleborn (1932), but it is an essential one; and further consideration shows that it raises this radical question: If a robot can be made which will copy vital actions, does it prove that the vital actions themselves are not instinctive—not due to a taxy? Put thus, the answer must be that it proves no such thing; the suggestion cannot be upheld. Now reasons have been given above for concluding that the hookworm larva possesses at its anterior end a sensory mechanism capable of stimulation by touch and thereby of inducing a true positive thigmotaxy. If this mechanism is also capable of receiving and transforming thermal impulses, it would supply the basis for positive thermotaxy. The matter must then be re-opened.

Just as in the effects of thigmotaxy, so in regard to the effects of heat, the larval movements observed are merely the ordinary movements activated by the stimulus. But are they directed by it? Both Fülleborn (1924, p. 158) and I (1930, p. 415), are agreed in the belief that very disturbing currents are set up by the local heating of water. Accordingly he asks why I did not repeat his agar experiments. They were in my programme, but illness broke off my work, and hitherto I have not found it possible to return to it. I had been the more anxious to test the point in that, at Fülleborn's demonstration at the London School of Hygiene and Tropical Medicine, in the spring of 1929, any interest by larvae in a hot point appeared to be absent. Further, it seems noteworthy that attraction of larvae to the cut edge of an agar plate, which Fülleborn (1932, p. 175) attributes to thermotaxy, only occasionally succeeds. On the other hand, it is obvious from his photographs (1932, figs. 10 and 11) that heat must be accepted as at times producing a directive influence on the normal movements of strongyloides and ancylostoma larvae which are on or in agar. Accordingly, their movement in water must also be influenced by it, and not merely by convection currents set up by heat; so that experiments which employ water are evidential here if used with discernment. One asks then: How powerful in general is the action of thermotaxy on infective nematode larvae, and what is its real significance for skin penetration? With *Monodontus trigonocephalus*, positive thermotaxy was invariably observed by Cameron (1923), although Hesse (1923) has noted exactly the opposite. Cameron concluded that Hesse's identification was erroneous—that he had not used the larvae which he thought he had. The alternative conclusion is not mentioned, namely, that thermotaxy cannot invariably be elicited among different batches of these larvae, a conclusion which at least falls in with the experience of Fülleborn, just noted, when dealing with strongyloides larvae. Schwartz (1924) used infective larvae of *Bustomum phlebotomum* and produced thermotaxy, but he expressly noted that when thigmotaxy had already been induced in these larvae by their contact with the surface layer at the edge of a drop of water, the stimulus of heat merely accentuated their thigmotaxic movements and produced no thermotaxy. The larvae remained pushing vigorously against the edge of the drop of water and were not attracted to the hot point. Thigmotaxy proved stronger than thermotaxy. These facts indicate that, in general, touch is the essential directing impulse for nematode larvae during the act of infection, heat being of great stimulative but of subordinative directive account. This conclusion is strengthened by the fact, to which Cameron (1923) first drew attention, that the presence of positive thermotaxy in a larva is no indication that it infects by penetration of the skin, a circumstance which has been shown to apply at least to *Monodontus trigonocephalus* (Cameron, in 1923), *Bustomum phlebotomum* (Schwartz, in 1924), *Thriconema* spp. [*sic.*] (Baudet, in 1925, as quoted by Mönnig, in 1930), *Trichostrongylus* sp. (Mönnig, in 1930), and *Obeliscoides cuniculi* (Alicata, in 1932). Larvae which show positive thermotaxy may then abstain from using it to enter their hosts. It appears to be a useless function in them, so that it seems unlikely to be of much importance in the act of infection by those larvae which use the skin route for that purpose. Passing to the particular, the evidence seems to point to thermotaxy as being present in hookworm larvae, as constituting a less effective directing impulse than thigmotaxy, and as thus being of less importance than thigmotaxy in the act of infection.

ABSTRACT AND CONCLUSIONS

Certain misconceptions having been cleared up, and it having been postulated that a taxy must express a vital act, it is concluded that hookworm larvae being obligatorily aquatic (a microscopic quantity of water in the liquid form sufficing for a microscopic animal), it is out of place to accept without clear evidence the presence of a hydrotaxy ; and it is pointed out that the evidence cited to that effect is not valid, the observed phenomena being adequately explicable as the result of purely physical forces. The same holds for the suggested rheotaxy. The evidence for the presence of gas taxies has failed to convince the writer. It is believed that chemical activation is real, and that it is unwise to reject summarily certain phenomena which, it is suggested, are possible indications that nourishment may reach a larva through its intact sheath and so may prolong its life. The taxy, which is held to be essential for the preservation of the individual and species by larval penetration of the skin of a host, is positive thigmotaxy; it is strictly defined as that induced by touch (*θίγμα*, touch) on the anterior larval face. It is suggested that a negative thigmotaxy, induced by touch on the larval body, adequately explains the phenomenon familiar in the waving larval tuft. The evidence for the presence of thermotaxy is believed to be good, the writer's previous mechanical interpretation failing to convince when pushed to its logical conclusion. In general, however, this taxy is erratic in action, is less potent than the thigmotaxy, and is seemingly useless in the life history of certain larval species which possess it but nevertheless abstain from using it for skin penetration. By all the evidence marshalled, thigmotaxy in its exact sense is the directive impulse essential for the penetration by hookworm larvae of man's skin, and so for the survival of individual larvae and of the species to which they belong.

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EXPERIMENTAL INFECTION OF MAN WITH A STRAIN OF *TRYPANOSOMA RHODESIENSE* APPARENTLY SUSCEPTIBLE TO NORMAL HUMAN SERUM *IN VITRO*

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PLATE I

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INTRODUCTION

Yorke, Adams and Murgatroyd (1930), from the comparative study of their six-year-old strain of *T. rhodesiense* and their one-year-old strain, ' Sherifuri K,' came to the conclusion that their old strain of *T. rhodesiense* had lost its resistance to human serum, as trypanosomes were no longer visible *in vitro* within two hours (although they did not establish the fact of all trypanosomes being killed, by sub-inoculating into rats); while their ' Sherifuri K ' strain, which they also thought to be *T. rhodesiense*, they considered to be on the way to becoming serum-sensitive, after one year's passage through five guinea-pigs. From this they concluded that *T. rhodesiense* would lose its serum resistance within two years of its being isolated from man, become incapable of infecting man, and so be changed in fact to *T. brucei*.

Corson (1932) proved that a strain of *T. rhodesiense*, maintained by direct inoculation in goats and sheep for 19 months, was still infective for man, as shown by personal inoculation, and was still transmissible by fly. Tested *in vivo* 3, 6 and 16 months after isolation, the strain was somewhat more susceptible to human serum than on first isolation ; while, 17 months after isolation, Adams had found that normal human serum, *in vitro*, killed the trypanosomes in 6 hours. A strain of *T. brucei*, however, which had been isolated four and a half years previously, though still transmissible by tsetse-flies, was non-infective to two Europeans. In a subsequent paper (1933), he showed that a Natal strain of *T. brucei*, isolated only one month previously, was also non-infective to four Europeans.

In a recent publication (Fairbairn, 1933), it was shown that the majority of 64 strains of *T. rhodesiense*, in the 1st rat passage from man, were resistant to human serum, when tested *in vitro* following the technique of Yorke, Adams and Murgatroyd (1930). Seventeen of these strains were sub-inoculated into rats, and the resistance to human serum was examined at each rat passage, for a variable number of passages, when it was found that the resistance was extremely variable, often disappearing by the 2nd passage to re-appear at subsequent passages.

THE EXPERIMENT

One particular strain, No. 26, having apparently become completely sensitive to the author's serum (' H.F.'), it was decided to test its infectivity for man.

The strain was isolated on the 21.2.32 from a Kibondo native, *aet.* 30 years, who gave a history of one month's illness. Table I shows in detail the resistance of this strain to a single serum.

It will be seen that trypanosomes were only once visible under the microscope at 8 hours (at the 3rd rat passage), and were seldom visible beyond 2 hours, while the rats sub-inoculated at the end of 24 hours' incubation only became infected at the 1st, 3rd, 5th and 8th rat passages. (In this connection the 1st rat passage is of particular interest.) The strain appeared to have lost its serum-resistance from the 9th rat passage onwards (i.e., 176 days after isolation).

At the 19th rat passage, when it had then been isolated 345 days, its serum-resistance was tested against 4 tubes of the author's serum. Immediately after the tubes had been put up, 1 c.c. of the Ringer-glucose-trypanosome mixture was inoculated subcutaneously into a rat, and 1 c.c. into the author's arm.

The rat became infected, with an incubation period of 4 days, and died in 15 days; while the author also became infected, fever starting at mid-day on the 7th day, and the blood being positive on the 8th day.

TABLE I

Showing the action, *in vitro* at 37° C., of human serum on consecutive rat passages of a strain, No. 26, of *T. rhodesiense*.

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture.
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
5.4.32	44	1st	9	44	1/8	H.F. (21)	88	58	7	2	0	0	—	0	Rat Neg. 30 days
								46	0	0	0	0	—	0	Rat + 7 "
29.4.32	68	2nd	5	25	1/11	H.F.	88	36	0	0	0	0	—	0	Rat Neg. 30 "
20.5.32	89	3rd	4	24	1/8	H.F.	52	44	38	4	2	1	0	0	Rat + 6 "
9.6.32	109	4th	5	21	1/10	H.F.	80	40	9	0	0	—	0	0	Rat Neg. 30 "
22.6.32	122	5th	4	14	1/20	H.F.	64	44	4 3 hrs.	0	0	—	0	0	Rat + 7 "
11.7.32	141	6th	4	20	1/27	H.F.	72	48	0	0	0	—	—	0	Rat Neg. 28 "
21.7.32	151	7th	4	11	1/8	H.F.	64	60	36	1	0	0	0	0	Rat Neg. 27 "
4.8.32	165	8th	4	15	1/4	H.F.	56	34	7	1	0	0	0	0	Rat + 14 "
15.8.32	176	9th	7	12	1/10	H.F.	80	76	7	0	0	—	0	0	Rat Neg. 35 "
24.8.32	185	10th	3	10	1/6	H.F.	72	84	12	0	0	—	0	0	Rat Neg. 28 "
5.9.32	197	11th	3	13	1/16	H.F.	104	84	18	0	0	0	0	0	Rat Neg. 26 "
21.9.32	213	12th	4	17	1/3	H.F.	28	36	9	0	1	0	0	0	Rat Neg. 38 "
—	—	13th	6	10		Blood clotted in pipette									

TABLE I—*Continued*

Date	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
		Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
17.10.32	239	14th	4	18	1/12	H.F.	76	80	18	1	0	0	0	0	Rat Neg. 29 days
31.10.32	253	15th	4	15	1/4	H.F.	72	88	24	0	0	—	0	0	Rat Neg. 60 „
26.11.32	279	16th	9	27	1/4	H.F.	76	104	16	0	0	—	0	0	Rat Neg. 34 „
5.1.33	319	17th	4	41	1/16	H.F.	136	112	5	0	0	—	0	0	—
—	—	18th	5	14		Rat died on 14th day.									
31.1.33	345	19th	5	13	1/15	*H.F.	120	80	21	0	0	0	0	0	Rat Neg. 30 „
						H.F.		88	20	0	0	0	0	0	Rat Neg. 30 „
						H.F.		92	20	0	0	0	0	0	Rat + 7 „
						H.F.		88	18	0	0	0	0	0	Rat Neg. 30 „
—	—	20th	4	13		Rat died on 13th day.									
25.2.33	370	21st	4	13	1/6	(19)	116	116	76	7	4	0	0	0	Rat + 7 „
						(22)		120	48	0	0	0	0	0	Rat Neg. 26 „
11.3.33	384	22nd	4	15	1/7	(22)	72	11	0	0	0	0	—	0	Rat Neg. 25 „
						(23)		36	1	0	0	0	—	0	Rat Neg. 25 „

* Trypanosome suspension inoculated into rat and human.

The demonstration of the loss by the strain of its serum-resistance is not quite conclusive, as one of the four tubes still contained enough living trypanosomes to infect a rat at the end of 24 hours; but according to the *in vitro* results one would have expected the strain to have become comparatively avirulent. That this was not so will be seen from a study of the clinical notes.

On the day that trypanosomes first became visible in the author's blood, ten rats were inoculated. Seven of these had incubation periods of 4 days, the other three of 5 days; eight of the rats died within periods of 17 to 25 days. Two guinea-pigs were also inoculated and showed incubation periods of 9 and 10 days, one of them dying in 56 days.

The 20th rat of the original strain (which corresponded to the human passage) having died before it could be examined, the 21st rat passage was examined together with the strain, in the 1st rat passage, recovered from the human. The result is shown in Table II.

It is of interest to compare the effect of serum on the trypanosome in rat No. 26/HF/1, with that of the original strain, also in the 1st rat passage from man. In both it will be seen that, in the case of one serum, no trypanosomes were visible after one hour, and yet the rat sub-inoculated at 24 hours became infected.

Rats Nos. 26/HF/1 and 26/HF/2 showed the same incubation period and were tested on the same day against the same serum, under identical conditions. The fact that the trypanosomes from the two rats showed such completely different re-actions to human serum, shows that the rats' tissues had a considerable influence on the inoculated trypanosome. In fact, it would appear that the trypanosomes in any strain of *T. rhodesiense* are not homogeneous, and that the number of serum-sensitive variants which arise in inoculated rats depends upon the hosts' tissues.

THE INFECTION IN MAN

On the 31.1.33, 1 c.c. of a Ringer-glucose-trypanosome mixture was inoculated subcutaneously in the left upper arm. For the next four days there was an occasional feeling of tension at the inoculation site on moving the arm, and if the skin were picked up between the finger and thumb it was slightly tender at one spot, but no lump

TABLE II

Showing the action of human serum on the 21st rat passage of strain No. 26, and on the 1st rat passage of the strain recovered from the human.

Date	Strain	No. of days since strain isolated	RAT			Rat blood diluted with Ringer-glucose	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours, rat inoculated with serum-trypanosome mixture
			Passage	Incubation period	No. of days since sub-inoculation			Start	1	2	4	6	8	12	24	
15.2.33	26	370	21st	4	13	1/6	(19)	116	116	76	7	4	0	0	0	Rat + 7 days
							(22)		120	48	0	0	0	0	0	Rat Neg. 26 ,,
	26/HF/1	17	1st	4	17	1/32	(19)	80	60	1	0	0	0	0	0	Rat + 10 ,,
							(22)		30	0	0	0	0	0	0	Rat + 5 ,,
	26/HF/2	17	1st	4	17	1/6	(22)	64	72	11	5	8	7	0	3	Rat + 5 ,,

was felt. By the fifth day, all feeling of tension had gone, the skin was not tender, and by the evening no trace of the inoculation was left.

On the evening of the sixth day, there was accidentally discovered a lump about the size of an almond, projecting about ¼ in. above the surface of the skin and covered with a faint violet tinge over an area about the size of a threepenny piece. The lump was not tender.

By the morning of the seventh day, the lump was larger and its borders more diffuse (it was now about 2 in. by 1 in.), very tender, the centre being a salmon-coloured patch (about the size of a sixpence) surrounded by a lilac areola ½ in. wide. There was an enlarged and tender gland in the axilla. At 10 a.m. the centre of the swelling was scarified, and the exudate was examined fresh for trypanosomes, but none were seen. By noon the temperature was 100·2° F., at 12.30 p.m. there was a rigor, and by 8 p.m. the temperature was 102·2° F.

Trypanosomes were first found in the blood on the afternoon of the eighth day, the experimental animals were inoculated, and an injection of ‘Bayer 205’ was given at 5 p.m.

The subsequent course of the disease may be summarized as follows:—

8.2.33.	7 0 a.m.	Temperature 102°.
	9 0 a.m.	Blood negative. (This, and all subsequent examinations, were of stained thick films).
	*3 0 p.m.	Temperature 103·2°. Blood positive—4 trypanosomes per 100 fields.
	5 0 p.m.	Photograph of injection site—Fig. 1. 10 rats and 2 guinea-pigs inoculated ‘Bayer 205,’ 1 gramme, intravenously—immediate vomiting.
9.2.33.	8 0 a.m.	Blood positive—averaging about 1 in 10 fields, mostly degenerated and little more than nucleus, blepharoplast and flagellum. Urine very faint trace of albumen. Dull headache, mostly occipital; general pains and stiffness.
	11 40 a.m.	Blood positive—about 1 to 6 or 7 fields, most of the trypanosomes degenerated but some well formed.
	4 0 p.m.	Temperature 102·7°. Photograph of injection site showing fully developed reaction—Fig. 2.

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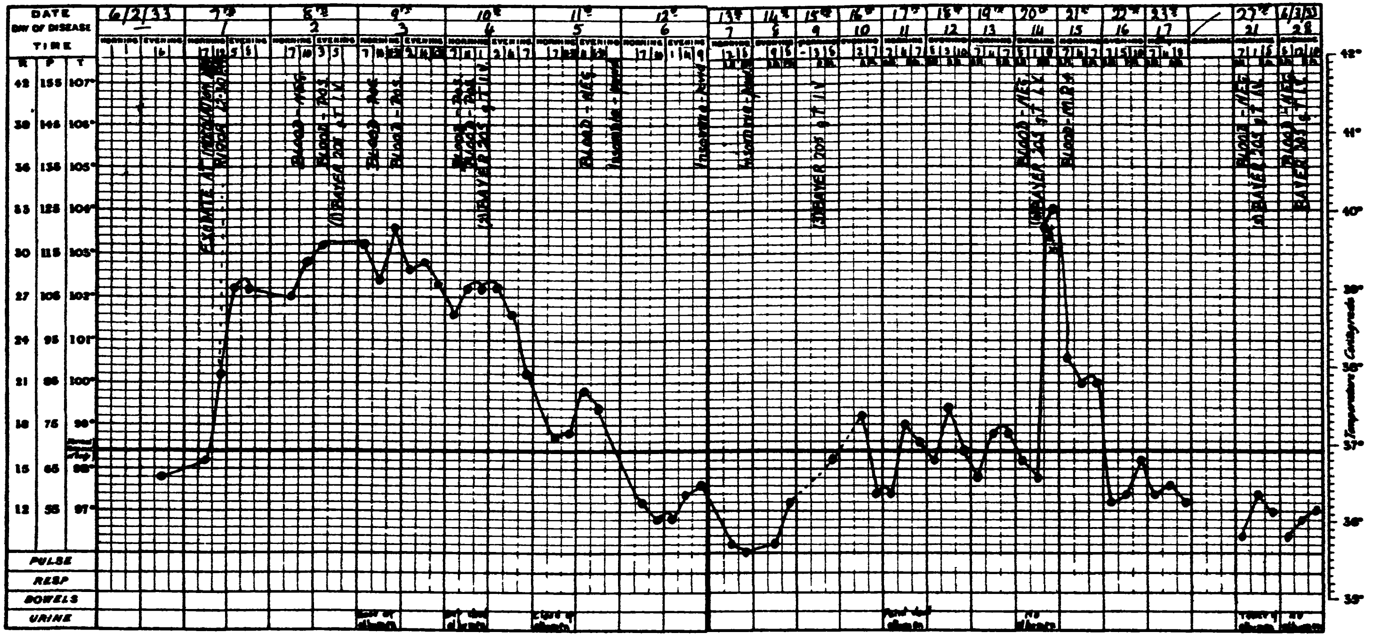
* For the early injections and the repeated blood examinations up to the 12.2.33, as well as for the photographs, I have to thank Dr. J. F. Corson, of the Trypanosomiasis Laboratory, Tinde, who attended me.


DATE
DAY OF DISEASE
TIME
MORNING
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R
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42
155
107°
39
145
106°
36
135
105°
33
125
104°
30
115
103°
27
105
102°
24
95
101°
21
85
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18
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15
65
98°
12
55
97°
PULSE
RESP
BOWELS
URINE
6/2/33
7th
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6/3/33
1
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12
13
14
15
16
17
21
28
EXCITATE AT INOCULATION
RIGOR 12:30
BLOOD - NEG
BLOOD - POS
(1) BAYER 205 1 G. I.V.
BLOOD - POS
BLOOD - POS
BLOOD - POS
BLOOD - POS
(2) BAYER 205 1 G. I.V.
BLOOD - NEG
Insomnia - head
Insomnia - head
Insomnia - head
(3) BAYER 205 1 G. I.V.
BLOOD - NEG
(4) BAYER 205 1 G. I.V.
BLOOD - M.R.A.
BLOOD - NEG
(5) BAYER 205 1 G. I.V.
BLOOD - NEG
BAYER 205 1 G. I.V.
Temperature Centigrade
42°
41°
40°
39°
38°
37°
36°
35°

10.2.33.	8 0 a.m.	Blood positive—scarce degenerated trypanosomes and numerous nuclear remains; no well-formed trypanosomes seen. Urine, definite cloud of albumen.
	10 45 a.m.	Temperature 102·2°. Blood positive—1 trypanosome seen in 10 minutes. No malaria parasites.
	12 45 p.m.	Temperature 102·2°. ‘Bayer 205,’ 1 gramme, intravenously—no reaction.
11.2.33	7 0 a.m.	Urine, distinct cloud of albumen, slightly less than yesterday.
	4 0 p.m.	Temperature 99·8°. Blood negative for trypanosomes and malaria. Insomnia at night and for two subsequent ones.
15.2.33.		Blood negative. ‘Bayer 205,’ 1 gramme, intravenously—no reaction.
17.2.33.		Urine, very faint cloud of albumen.

From the 16th to the 19th, there was a persistent afternoon temperature of between 99° and 99·4°. A gland in the left axilla was swollen and tender, and a lump was still present at the site of the inoculation.

20.2.33.	8 0 a.m.	Temperature 98·2°.
	1 0 p.m.	Temperature 97·8°. Blood negative. Urine, no albumen. ‘Bayer 205,’ 1 gramme, intravenously.
	3 0 p.m.	Rigor.
	5 30 p.m.	Temperature 103·8°.
	9 0 p.m.	Temperature 104°.
21.2.33.	7 15 a.m.	Temperature 100·6°.
	8 0 a.m.	Blood, very scanty malaria parasites. Urine, slight albumen.
	4 0 p.m.	Temperature 100°. Slight itchiness of skin of forehead and jaws began.
27.2.33.		Blood negative. Urine, very faint trace of albumen. ‘Bayer 205,’ 1 gramme, intravenously—no reaction.
28.2.33.		Urine, very faint cloud of albumen.
6.3.33.		Blood negative. Urine, no albumen. ‘Bayer 205,’ 1 gramme, intravenously—no reaction.
7.3.33.		Urine, no albumen.

Treatment was not interrupted, in spite of the presence of albumen in the urine, and further injections of ‘Bayer 205’ did not appear to aggravate the kidney condition. This is in line with what we have found in the routine treatment of large numbers of natives.

On the afternoon of the 21st (i.e., the day after the 4th injection of ‘Bayer 205’), there developed an itchy condition of the face and forehead. On the succeeding days, this itchiness spread to the chest, abdomen, back and head, without there being any obvious rash to account for it. Presumably it was due to the 'Bayer 205.' This skin condition did not subside until March 1st, i.e., it lasted 8 days. The 5th injection of 'Bayer 205' on the 27th did not aggravate the condition. Dr. Corson informs me that an itchy papular rash developed in one of his Ikoma cases: this woman had had 6 grammes of 'Bayer 205' in 28 days, and the rash appeared 3 days after the last injection and lasted 17 days. A somewhat different skin condition, following 'Bayer 205,' has been described by Maclean (1928).

The lump at the injection site did not disappear until the 27th, and the axillary gland, though still palpable, was no longer tender on that date.

The first photograph shows the condition at the inoculation site on the 8.2.33, while the second photograph, taken on the 10.2.33, shows the fully developed reaction. The central dark-red spot was surmounted by a number of very small vesicles; and, following the disappearance of this reaction under treatment, there was a very slight scaly desquamation.

It will be seen from the clinical history that the infection was anything but mild, and was not what one would expect from a study of the *in vitro* results.

DISCUSSION

In the paper by Yorke, Adams and Murgatroyd (1930), they remark: 'It seems to us incredible that our laboratory strain of *T. rhodesiense* which, as we have shown, is killed by normal human serum, or plasma, *in vitro* at 37° C. within a few hours, . . . could infect man'; and later: 'The pronounced sensitiveness to normal human serum of *T. rhodesiense* which has sojourned for some time . . . in vertebrate hosts other than man, provides almost overwhelming evidence that normal human beings cannot become infected with such strains.' In the light of the present experiment both these assumptions appear unfounded.

Yorke and his co-workers, in their work, did not sub-inoculate rats with the serum-trypanosome mixture at the end of 24 hours' incubation, and so do not know if their strains had really been killed by the serum. And, as this experiment has shown, even the fact of the sub-inoculated rats not becoming infected over many passages is no guarantee that the strain is non-infective to man.

Since the discovery of 'Bayer 205,' and its proved efficacy in early infections, experiments on man have lost their danger; and it appears desirable that the infectivity for man of the old strains of *T. rhodesiense* in European laboratories should now be tested by direct human inoculation, as only thus can it be proved whether they are infective or not.

Their statement 'of the striking relative immunity of man . . . to all members of the genus TRYPANOSOMA, except *T. gambiense*,' also requires comment. During eight years' work on sleeping sickness in all the infected areas of Tanganyika both epidemic and endemic, the author has been bitten many times by both *G. morsitans* and *G. swynnertoni*; his serum *in vitro* has been shown to be of average trypanocidal power, and yet he became infected immediately he was inoculated with *T. rhodesiense*—and with a strain, at that, which had not been in contact with human serum for nearly a year, and which appeared to have become sensitive to his serum, when tested *in vitro*.

At the height of the Kahama epidemic, in March and April, 1929, 1,373 salivary glands of *G. morsitans* were examined, using the technique of Lloyd and Johnson (1924), and 2 glands were found infected, giving an infectivity rate of 1·4 per thousand. In March and April, 1931, the same area was revisited, 18 months after the population had been completely evacuated, and in 2,189 salivary glands examined 2 were found infected, giving an infectivity rate of 0·9 per thousand. The main food supply of *G. morsitans*, as shown by Johnson and Lloyd (1923), is antelope blood, and the usual trypanosome met in the salivary glands will be *T. brucei*: the difference between the 1929 and 1931 figures has no statistical significance, but they give an indication of how low the infectivity rate of *G. morsitans* with *T. rhodesiense* must have been, which in itself is enough to explain the sporadic character of the disease, where the population is only in intermittent contact with the fly. But the results in Kahama District, where there were 3,069 microscopically proved cases during 1928 and 1929 (including 3 Europeans who were living in the area), and in Kibondo District, where there were 1,142 cases during 1932, shows that man has no great resistance to the disease, provided that the contact with tsetse-fly is close enough.

With reference to the point of whether man's immunity or susceptibility to infection is bound up solely with the trypanolytic power of his serum, the following observations are of interest :—

1. Mesnil and Leboeuf (1912) showed that a baboon's serum had no action on *T. gambiense in vivo*, and yet the baboon was unable to be infected.

2. Regendanz (1932), by injecting *T. gambiense* into the cerebro-spinal fluid of a baboon, has produced a chronic infection of its nervous system, without producing a blood infection.

From the above it would appear that the baboon's whole-blood has a trypanocidal action on *T. gambiense* which is lacking in its serum and its cerebro-spinal fluid.

3. Mesnil and Leboeuf (1912) made a strain of *T. brucei* in mice resistant to 2 c.c. of baboon serum, and yet were unable to infect the baboon with this strain. They concluded that the properties of a serum form only a small part of the natural means of defence of the animal which furnishes the serum.

4. Collier (1924) made a strain of *T. brucei* in mice resistant to 2·5 c.c. of human serum, but he himself and four other people were not infected when subsequently inoculated with the strain. He considered that the pathogenicity of trypanosomes has nothing to do with the degree of resistance to the serum of the species of animal concerned.

5. Corson (1933) found that the serum of resistant rats and guinea-pigs had no trypanocidal action *in vitro* on *T. brucei*.

On the other hand there are :—

6. The fact that Corson was infected with a strain of *T. rhodesiense*, which both *in vivo* and *in vitro* had been shown to be susceptible to human serum.

7. And the present experiment.

From the above it would thus appear that unshed human and baboon blood has a different trypanocidal action from that of *serum*, and that tests of the trypanocidal action of *sera* are not necessarily indicative of what may take place in the human or animal body.

If one's whole defence were dependent upon the trypanolytic action of *serum*, it is extraordinary that *T. gambiense*, which is completely resistant to human serum, should only produce a chronic infection in man, while *T. rhodesiense*, which is comparatively susceptible to human serum (even on first isolation), should produce an acute, rapidly fatal disease. There must be some other factor, or lack of one, in unshed blood, which produces these results.

SUMMARY

A strain of *T. rhodesiense,* isolated from man 345 days previously and maintained in rats by direct inoculation, had become very susceptible *in vitro* to normal human serum from the 9th rat passage onwards, i.e., 176 days after isolation. This strain was nevertheless still infective for man, as proved by human inoculation, at the 19th rat passage.

It is apparent that the *in vitro* technique used is not able to indicate whether a strain has lost its infectivity for man or not.

Man is not really immune to *T. rhodesiense,* given the necessary contact with infected tsetse-fly.

From a study of the literature, it is suggested that the trypanolytic power of serum, or the lack of it, is no indication of the power of the body to deal with trypanosomes.

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EXPLANATION OF PLATE I

Fig. 1. 8.2.33, the eighth day after the inoculation of trypanosomes, showing the skin reaction the day the blood first showed trypanosomes, and just before the first injection of 'Bayer 205.'

Fig. 2. 9.2.33, the ninth day, showing the fully developed reaction.

(Photographs by Dr. J. F. Corson.)

PLATE I

Fig. 1

Fig. 2

ANOPHELES DEMEILLONI SP. N., A NEW NAME FOR *ANOPHELES TRANSVAALENSIS* OF AUTHORS

BY

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(*Received for publication* 11 *May*, 1933)

Recently, when reading Carter's (1910) description of *A. transvaalensis*, it was noticed that both the text and the illustration of the wing clearly indicated the presence of a small pale interruption in the third dark area on the first vein. As the absence of such a spot* is a constant character of the species commonly known as *A. transvaalensis*, I mentioned the point to Dr. F. W. Edwards, who kindly examined the type that is in the British Museum and informed me that such an interruption is present in the wings of the type, and, moreover, that the illustration given was evidently made from Carter's other specimen. Later, Dr. Edwards showed me the type, and we both agreed that the banding of the palps did not differ from the condition sometimes seen in specimens of *marshalli*, the distal dark band being only slightly longer than the subapical pale band. The hind tarsi showed distinct whitish apical banding as broad and distinct as in *marshalli*. There are very few other characters that are of significance in deciding what Carter's type is. The markings of the wing field vary considerably in both *marshalli* and the species known as '*transvaalensis*'; the wings of Carter's specimens are, however, somewhat paler than is usually the case in Transvaal specimens of the latter species, and in this respect would agree with some South African specimens of *marshalli*.

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* It seems to be a fact that when this spot is normally absent in a species it is invariably absent. To be quite certain on this point, however, I asked Dr. De Meillon whether he had ever seen a '*transvaalensis*' in which it was present, and he informed me that the spot is always absent in S. African specimens. Mr. Gibbins had also found it constantly absent in specimens bred from larvae in Uganda.

It thus appears that Carter's specimens do not differ from known specimens of *A. marshalli*, but that, in possessing the pale interruption in the third dark area on the first vein, they definitely differ from the species hitherto referred to as *transvaalensis*. That *marshalli* is an entirely distinct species from '*transvaalensis*' of authors has been demonstrated by De Meillon (1931) who showed that, on the character of the larval pleural bristles, the two species fall into different groups, the former being a typical member of the *Myzomyia* group, the latter having the character of the *sergenti* sub-group. Recently, Symes has found that the larva of the form which I described as *A. marshalli* var. *keniensis* is of this latter type and is very similar indeed to that known as *transvaalensis*. It is obvious, therefore, that, in spite of its *marshalli*-like palps, *keniensis* is more closely related to '*transvaalensis*' of authors, which it further resembles in lacking the pale interruption in the third dark area of the first vein. Consequently, *Anopheles keniensis* must now be regarded as a distinct species.

A. transvaalensis Carter must, therefore, be placed as a synonym of *A. marshalli* and a new name be given to the species usually identified as *A. transvaalensis*. This species is here named in honour of Dr. B. De Meillon.

ANOPHELES (MYZOMYIA) DEMEILLONI sp. n., type form

This is the species mainly or entirely indicated as *Anopheles transvaalensis* by the following authors :—Edwards, *Bull. Ent. Res.*, Vol. 3, p. 244 (1912) ; Christophers, Prov. List and Ref. Cat. of *Anoph.*, p. 94 (1924) ; Evans, *Anoph. Trop. S. Afr.*, p. 33 (1927) ; Symes, *Kenya and E. Afr. Med. Jl.*, Vol. 7 (1927) ; Symes, *Rec. Med. Res. Kenya* (1932) ; De Meillon, *Ann. Trop. Med. & Parasitol.*, Vol. 27, p. 83 (1933).

An account of the species is to be given in the forthcoming monograph on African mosquitoes, in which the writer is collaborating with Dr. Edwards and Mr. Hopkins, and certain distinguishing characters in the female, larva and pupa will be dealt with in a forthcoming paper in this journal written in collaboration with Dr. De Meillon. It is, therefore, unnecessary to give more than a brief diagnosis of the species here.

FEMALE (fig. 1). Medium sized, with the characters of the *Myzomyia* group.

Head. Pale scales white, occasionally creamy white; those next to dark scales sometimes slightly ochraceous. Palpi with three pale bands, a very narrow one at the apex of the second segment; a broader one of variable length occupying the tip of the third and base of the fourth segments and a broad apical band including the whole of the last and tip of the fourth segments; intervening dark band longer than subapical pale band, usually at least one and a half times and often three times its length or more. Palpal index about 0·4.

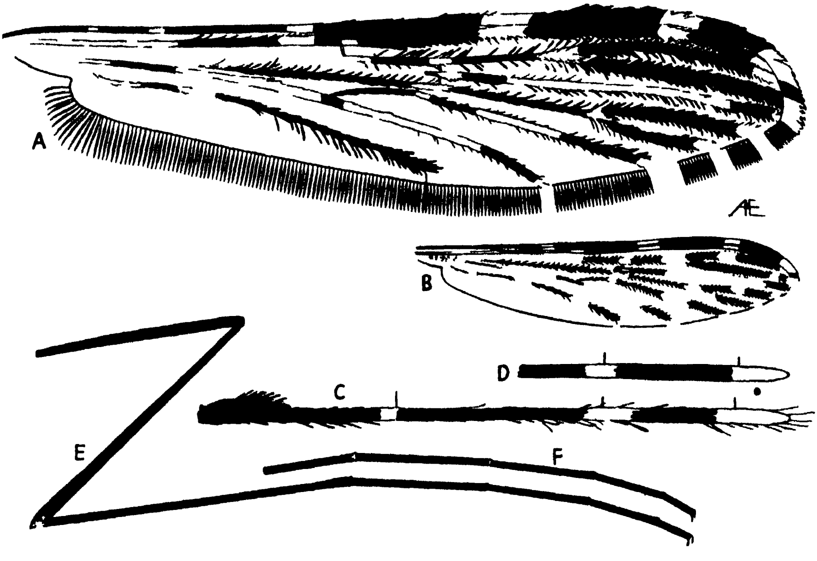

FIG 1. *Anopheles demeilloni* sp n A and B —Wings showing variation in markings, B more diagrammatic and to smaller scale than A C ♀ palp and D.—Outer half of palp showing variation. E.—Hind leg F.—Hind tarsi of specimen showing indications of apical banding.

Pharyngeal armature. The structure conforms to that of Barraud and Covell's Class D, which includes the species of the *Myzomyia* group. The rods are of the type with broad bulbous bases, and the filaments of both rods and cones project at a considerable angle to the main plane of the pharynx.

Thorax. Mesonotum sparsely clothed with scales which are white or greyish white, at least anteriorly. Scales almost always of the very narrow curved type, often hair-like on posterior half

Abdomen without scales.

Legs (fig. 1, E and F). Tibial knee spots distinct. Fore and mid-tarsi sometimes with narrow but fairly distinct apical pale rings on first two or three segments. Hind tarsi usually entirely dark but, as noted by De Meillon (1931), sometimes with very narrow, indistinct apical rings on first two to four segments.

Wings (fig. 1, A and B). Four main pale costal spots usually well developed but small; base of costa with one or two pale interruptions or entirely dark. First vein without pale interruption in third dark area; accessory sector spot present. Third vein usually mainly pale but dark areas sometimes extensive and pale area occasionally less than half the length of the vein. Second and fourth veins predominantly dark, branches with or without a pale spot in addition to those at fork and apex. Upper branch of fifth vein very variable, stem and lower branch largely pale with dark spot constantly present at the fork. Sixth vein commonly with outer half or more entirely dark, but sometimes with a pale spot. Fringe spot at apex of sixth vein present or absent.

Wing length. Usually about 3·6-4·2 mm., sometimes longer in East African specimens.

MALE. *Palps.* Club with three pale spots present but sometimes greatly reduced.

Terminalia. The following specific characters may be noted :—Harpagones with one outer and one or two inner accessory hairs which are usually more than half the length of the apical hair. Phallosome with about five to eight pairs of leaflets.

SOUTH AFRICA :—Tzaneen, Transvaal, 4.11.1932, Dr. B. De Meillon, Type ♂ and ♀ (crevices), paratypes, 5 ♀♀; Zululand; Southern Rhodesia.

EAST AFRICA :—Nyasaland; Kenya; Uganda. BELGIAN CONGO :—(Luofu, L. Edward, Dr. J. Schwetz).

Types presented to the British Museum.

Variation. Specimens from East Africa differ from South African material as follows. Size more variable, specimens occasionally attaining a wing length of about 4·6 mm. (South African specimens of the type form are not known to exceed about 4·2 mm.). Basal portion of costa usually entirely dark and very rarely with more than one pale interruption. Fourth vein very commonly as in fig. 1, B, especially in certain localities, including Nairobi.

As pointed out by Mr. Gibbins, in one otherwise normal female among a series of this species bred out from isolated larvae which he collected at Fort Portal, Uganda, the distal pale palpal bands were very broad. The intervening dark band was only just equal to the subapical, and shorter than the apical, pale band, the palps thus resembling those of some specimens of the type form of *marshalli*. A distinct South African variety of this species will be described in the forthcoming paper in collaboration with Dr. De Meillon.

ACKNOWLEDGMENTS

To Dr. F. W. Edwards I am greatly indebted for the loan of material of this species in the collections of the British Museum. This includes many specimens taken in Kenya by Mr. C. B. Symes, who has also kindly presented material of adults and larvae to this School. Mr. G. H. E. Hopkins sent us the valuable series of adults with larval and pupal pelts, from Fort Portal, Uganda, collected by Mr. E. G. Gibbins, whose notes on the characters of the species in that region have been of great assistance. I have also to acknowledge the indispensable help of Dr. B. De Meillon in kindly supplying both material of and information about the species in South Africa.

In conclusion, I must express my regret at the necessity for renaming a species which has been known for so long as *A. transvaalensis*. It is greatly to be hoped that the suggestion put forward by Balfour-Brown (1933) may lead to the establishment of an effective means of conserving such a generally accepted name, and that in future this course will be adopted by the leading systematists, at least in the case of species which are of any general, economic or medical interest.

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NOTES ON *ANOPHELES DEMEILLONI (TRANSVAALENSIS)* AND *ANOPHELES GARNHAMI* IN SOUTH AFRICA, WITH DESCRIPTIONS OF NEW VARIETIES OF THESE SPECIES

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(*Received for publication* 11 *May*, 1933)

In a previous paper in this journal, Evans has redescribed under the name *Anopheles demeilloni* the species mainly or entirely referred to as *A. transvaalensis* by almost all earlier writers dealing with South and East African anophelines. *A. transvaalensis* Carter (1910) was shown to be a synonym of *A. marshalli* Theobald.

It has recently been found that four different anophelines have up to the present been included under *demeilloni* in South Africa. They are as follows :—

1. *A. demeilloni* Evans, type form.
2. *A. demeilloni* var. *carteri* var. nov.
3. *A. garnhami* Edwards, type form.
4. *A. garnhami* var. *walshi* var. nov.

A comparison between the characters of the adult, larval and pupal stages of these forms has been made, and it is found that they may be distinguished as follows :—

A. DEMEILLONI Evans, type form

A. transvaalensis (in part, at least) of former authors,
except Carter (1910).

The following definition applies mainly to South African specimens.

FEMALE. A medium-sized mosquito ; wing length normally about 3·6 to 4·2 mm. ; average about 3·8 (fig. 1, B).

Palps with 3 pale bands ; no tendency to form 4 bands.

Mesonotal scales. Scaled area of mesonotum sparsely clothed with pointed scales (fig. 2, A-C), which are typically very narrow; breadth index about 18, and often hair-like (fig. 2, D) especially on posterior half. Exceptionally, the scales are distinctly wider (fig. 2, E-F), breadth index 8-13. Scales white or greyish-white, at least anteriorly.

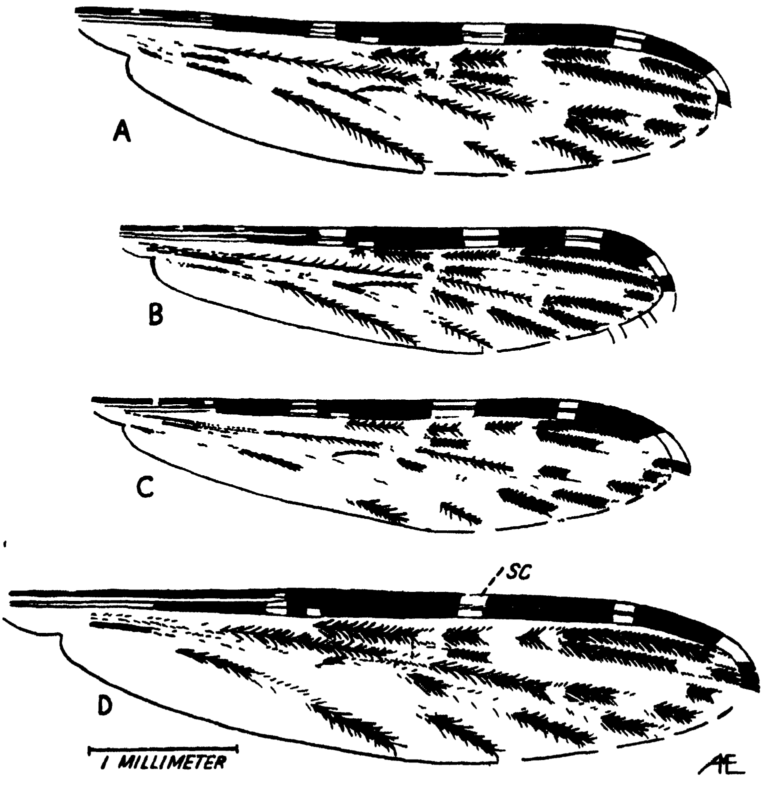

FIG. 1. Wings, diagrammatic A.—*A. demeilloni* var. *carteri* B—*A demeilloni* type form C.—*A. garnhami* var. *walshi*. D.—*A. garnhami* type form, SC—subcostal pale spot on costa

Wing (fig. 1, B). Costa always with at least 1 basal pale spot, usually there are 2 such spots (spots commonly absent in East African specimens). Branches of second vein either with or without pale spots; both sometimes entirely dark in the same specimen. Fifth vein with a dark spot on the lower branch at the fork; this extends along the upper branch as a larger spot. Sixth vein usually

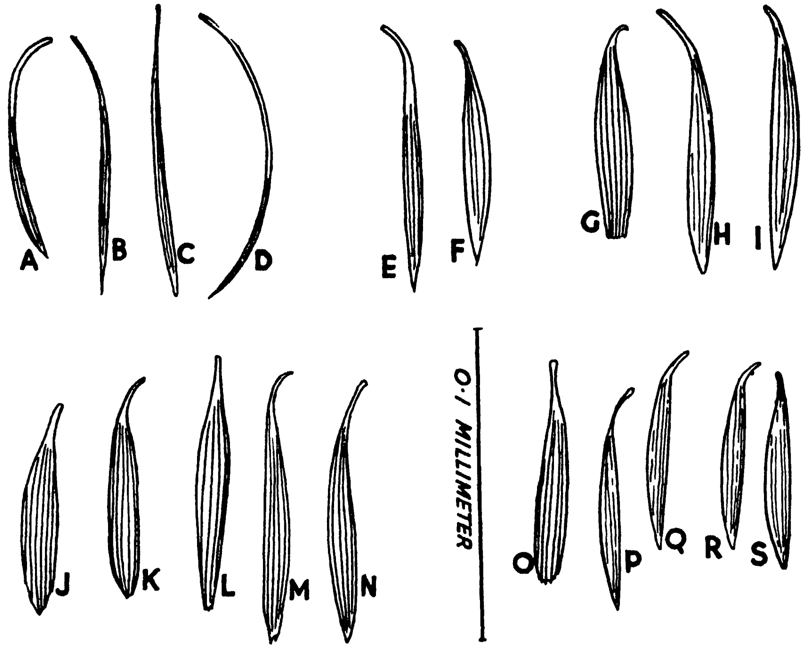

FIG. 2. Mesonotal scales. *A. demeilloni* type form :—A.-D.—typical, E. and F.—abnormally broad ; *A. demeilloni* var. *carteri* :—G, H. and I. ; *A. garnhami* type form :—J.—from S. African female ; K.-N.—from East African females ; *A. garnhami* var. *walshi* :—O.-S. Drawn by the authors.

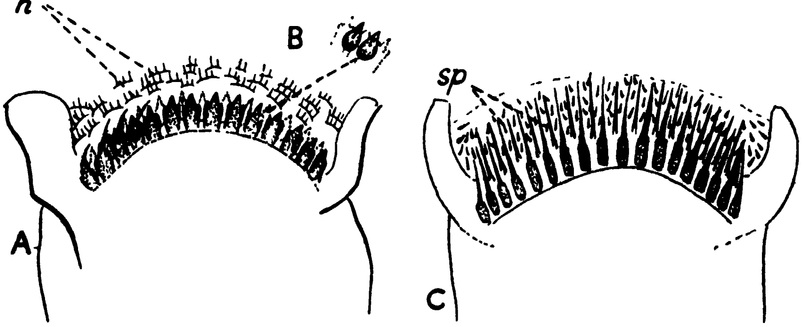

FIG. 3. Pharyngeal armature. A.—*A. demeilloni* type form, rods indicated by dotted lines, filaments shown only on four cones at left. B.—two rods of same. C.—*A. garnhami* var. *walshi*, filaments of all cones and rods shown. *h*.—hairs and *sp*.—spicules of pharyngeal ridges. Drawn by the authors.

with outer half all dark, but this dark area sometimes interrupted by a pale spot.

Pharynx (fig. 3 A, B). With 14 ± 2 pairs of rods and cones. Cones stout and appearing short when pharynx mounted dorso-ventrally. Pharyngeal ridges with rows of fine short microtrichia as in most other *Myzomia* (Barraud and Covell, 1928).

MALE. *Hypopygium* (fig. 4, A-C). Harpago with club of normal shape; outer and 1 or more inner accessory hairs present. Phallosome with 5 to 8 leaflets on each side.

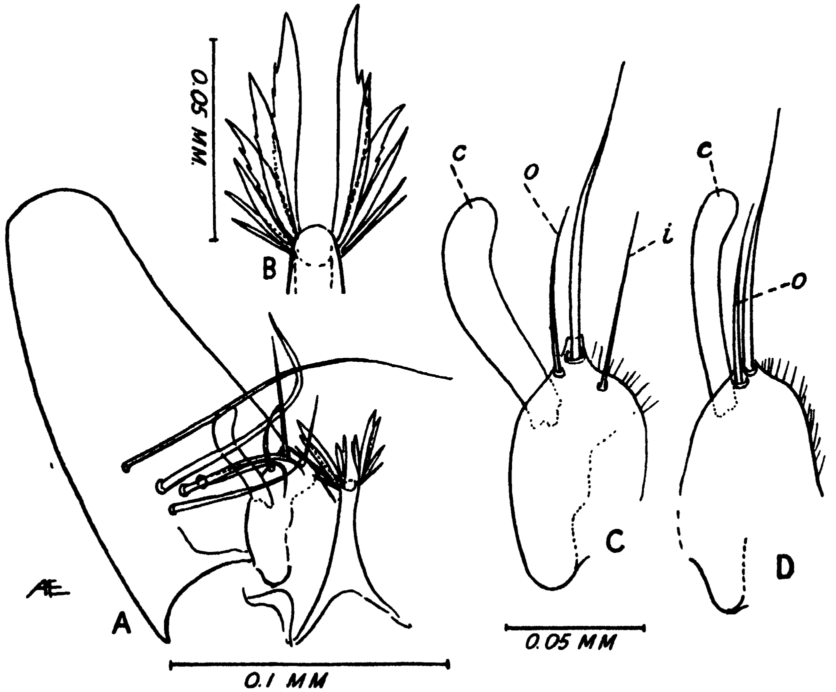

FIG. 4. Male hypopygium. *A. demeilloni* type form :—A.—coxite and phallosome (phallosome and hairs of harpago foreshortened); B.—phallosome leaflets; C.—harpago. *A. garnhami* var. *walshi* :—D.—harpago. c.—club; i.—inner, o.—outer accessory hair.

LARVA. *Thoracic palmate hair* (fig. 5, A), with narrow blade-like leaflets.

Post. ventral mesopleural hair (longer of the 2 short hairs), branched or split, at least bifid, and relatively shorter than in *garnhami* and its variety.

Typical abdominal palmate hairs, i.e., those of segments IV and V (fig. 5, B, C). Leaflets with moderately long or long filaments* with linear termination. The filaments are usually about half the length of the blade or more, but may be two-fifths or, exceptionally, less than one-third; they are usually abruptly narrowed to form the hair-like termination which is normally at least one-sixth the length of the blade and commonly one-third or more.

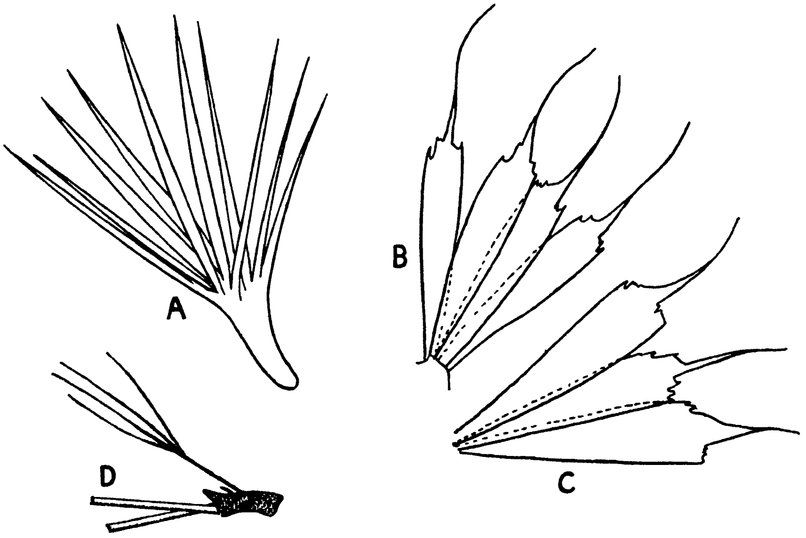

FIG. 5. Details of fourth stage larva of *A. demeilloni* type form. A.—Thoracic palmate hair. B. and C.—leaflets of typical abdominal palmate hairs. D.—base of mesopleural hair group showing branched posterior ventral. D. to smaller scale than A. and C. Drawn by the authors.

Inner caudal bristles with several branches hooked at tip.

PUPA (fig. 6, A and C). *Bristle C* on abdominal segments V and VI simple, and usually about equal to or slightly more or less than the length of the succeeding segment.

Bristle A on segment VIII with relatively numerous branches, 9 to 15, usually about 12.

Hair IV on segments VI and VII with at least 5 branches.

Apical bristles of paddles. Long and with double hook characteristic of *Myzomyia* (Senevet, 1931).

DISTRIBUTION IN SOUTH AFRICA. Widespread in North and North-Eastern Transvaal; also found in Zululand (collected by Dr. A. Ingram) and Southern Rhodesia, Leeson (1931).

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* The filament is here defined as the portion of the leaflet beyond the last serration.

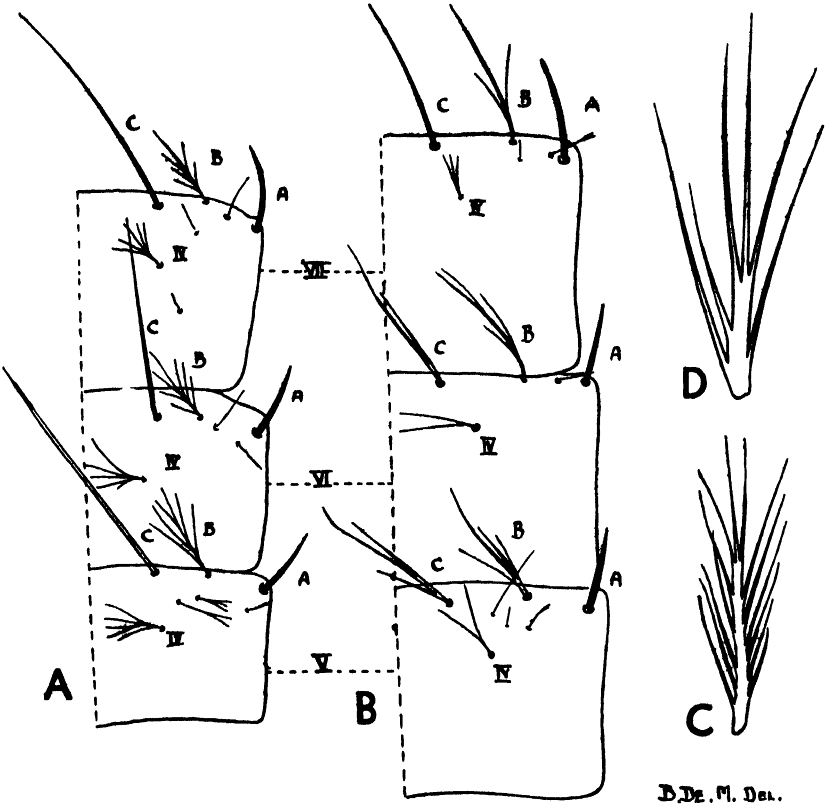

FIG. 6. A.—abdominal segments V-VII of pupa of *A. demeilloni* type form. B.—the same of *A. garnhami* var. *walshi*. C.—bristle A of segment VIII of *A. demeilloni*. D.—the same of *A. garnhami* var. *walshi*. The small capitals and numerals indicate the important bristles and hairs.

A. DEMEILLONI var. *CARTERI* var. nov.

A. transvaalensis De Meillon (in part).

FEMALE. On the average larger than the type form; average wing length about 4·4 to 4·5 mm.

Palps with either 3 or 4 pale bands, the latter condition very common.

Mesonotal scales (fig. 2, G-I). Scaled area densely clothed with rather narrow white scales which are much broader than those in typical *demeilloni*, the narrowest having a breadth index of about 9, several being broadly truncated with index 6. Other characters including wing markings, colour of pale scales, structure of pharynx and ♂ hypopygium as in type form.

LARVA. Very similar to that of the type form, but: *Thoracic palmate hair* either with narrow blade-like leaflets as in the type form or represented by a branched hair.

Typical abdominal palmate hairs (fig. 7, B, C). Leaflets very variable in width and sometimes much narrower than is usual in the type form. Filaments always shorter than in typical specimens of the type form and either without or with very short linear terminations.

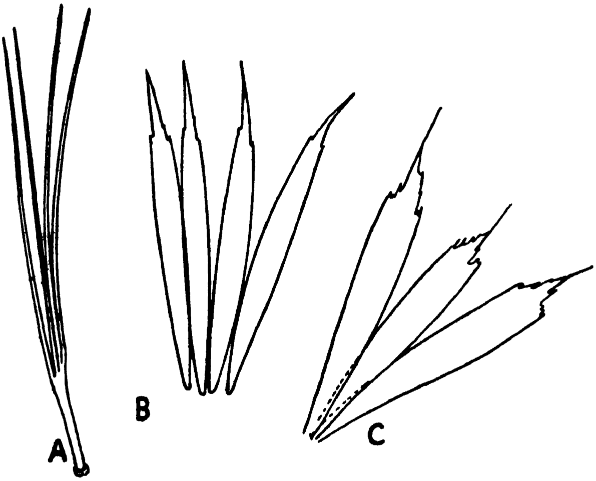

FIG. 7. *A. demeilloni* var. *carteri*, larva. A.—thoracic palmate hair. B. and C.—leaflets of typical abdominal palmate hairs. Drawn by the authors.

Inner caudal bristles with branches rather less markedly hooked than in the type form.

PUPA. Similar to that of type form.

DISTRIBUTION. Johannesburg, Transvaal, collected by one of the writers (B. De M.), including cotype ♂♂ and ♀♀; Weenen, Natal (specimens in collections of the British Museum).

Variation. In a specimen in the collections of the British Museum, taken at Estcourt, Natal, September to October, 1899, by Sir G. A. K. Marshall, the mesonotal scales were on the whole much narrower than in typical examples of the variety.

A. GARNHAMI Edwards, type form

Specimens referable to this species were collected by one of us (B. De M.) in South Africa, in 1930, but were then regarded as variants of *demeilloni* (*transvaalensis*). On re-examining the collections of the South African Institute for Medical Research, 5 ♀♀ of this form were discovered.

The adult stage of the species was described in detail by Edwards (1930), who noted the external differences separating it from *demeilloni* (*transvaalensis*). Later the pharyngeal characters were studied by one of us (A.M.E.) and a marked peculiarity noted, which distinguishes *garnhami* from all other *Myzomyias* in which those characters are known. We now find that the character of the pharyngeal armature affords a reliable distinction between *A. garnhami* and its var. on the one hand, and *A. demeilloni* and var. *carteri* on the other. The characters of the hypopygium appear to give a morphological distinction in the ♂♂.

Characters found to be of value in distinguishing this form may be summarised as follows :—

FEMALE. Larger than the other 3 anophelines here dealt with ; wing length usually from 4·8 to 5·5 mm. (fig. 1, D).

Palps normally with 3 pale bands ; tendency to 4-banded condition very rare.

Mesonotal scales (fig. 2, J-N). Scaled area densely clothed with rather narrow scales, which are much broader than in typical *demeilloni* but of about the same width or broader than those of var. *carteri* ; many of scales in South African specimens with breadth index 6. In East African specimens the scales tend to be somewhat narrower. The scales have a distinctly yellowish tinge, especially towards the sides of the scaled area anteriorly.

Wing (fig. 1, D). Costa almost always without a basal pale spot. Pale costal spots very small and any one except the subcostal (SC) may be absent. Fifth vein without dark spot on stem or lower branch at the fork.

Pharynx with 19 ± 2 pairs of narrow rods and cones. Pharyngeal ridges (as in fig. 3, C) very well developed and bearing, instead of the usual fringe of short, delicate hairs, a single stout spicule. Those immediately behind and alternating with the rods have processes somewhat resembling the filaments of the rods but shorter. When well displayed, at least 3 alternating rows of ridges can be seen behind the first, the spicules becoming progressively smaller. The spicules can be readily seen in unstained preparations.

MALE. *Hypopygium.* Harpago (as in fig. 4, D) with club long and very slender; inner accessory hair absent. Phallosome with not more than 4 leaflets on each side.

LARVA. The larva was discovered by Symes, who described it in 1932, and we have examined specimens collected by this observer and also larval material received from Mr. Hopkins and Mr. Gibbins in Uganda. Characters distinguishing the larva from that of *A. demeilloni* are:—

Thoracic palmate hair (fig. 8, A), very rudimentary, usually with about 6 or 7 extremely narrow leaflets, which sometimes arise from widely different levels.

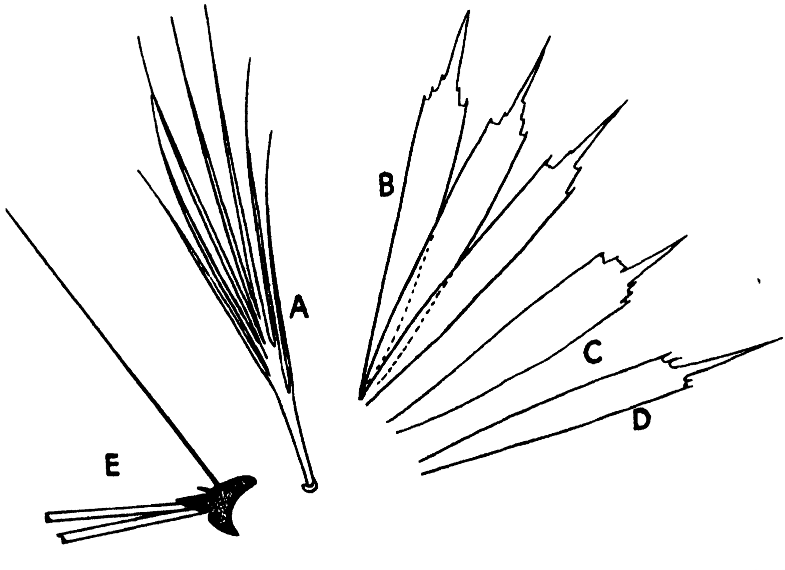

FIG. 8. *A. garnhami* type form, larva. A.—thoracic palmate hair. B., C. and D.—leaflets of typical abdominal palmate hairs. E.—base of mesopleural hair group, showing simple posterior ventral. E. to smaller scale than A.-D. Drawn by the authors.

Posterior ventral mesopleural hair (fig. 8, E). Nearly always simple; relatively longer than in *demeilloni* and its variety.

Typical abdominal palmate hairs (fig. 8, B-D). Leaflets with filaments relatively shorter on the whole than in typical *demeilloni* and without or with very short linear termination.

Inner caudal bristles with none of branches definitely hooked at tip.

PUPA. This was described by Evans (1932), and the main differences from *demeilloni* are now found to be as follows :—

Bristle C on abdominal segments V and VI with 2 to 4 branches (Evans, 1932, includes the main stem in figures given).

Bristle A on segment VIII coarse and with smaller average number of branches, about 7 to 9.

Hair IV on segments VI and VII with 1 or 2 branches.

Apical bristle of paddle short and with at most a slight tendency to the usual hooked shape.

DISTRIBUTION IN SOUTH AFRICA. In foothills of Drakensburg Mountains, near Leydsdorp, Transvaal (2,000 to 3,000 ft.).

A. GARNHAMI var. *WALSHI* var. nov.

FEMALE. Smaller than the type form, wing length about 4 mm. (fig. 1, C).

Palps commonly with 4 pale bands.

Mesonotal scales. Mesonotum densely clothed with rather narrow yellowish scales many of which are rounded distally with breadth index 7.

Wing (fig. 1, C). Costa with 2 basal pale spots. As in the type form of *garnhami*, the other pale costal spots more reduced than in South African *demeilloni*, but little tendency, if any, to complete absence of one or other of these.

Pharnygeal armature and ♂ *hypopygium* (fig. 4, D) as in the type form of *garnhami.*

LARVA. Very similar to that of the type form of *garnhami.*

Thoracic palmate hair commonly an unmodified branched hair (fig. 9, A).

Post. ventral mesopleural hair as in *garnhami.*

Typical abdominal palmate hairs usually much as in *garnhami* but filament sometimes longer, nearly half the length of the blade in one larva (fig. 9, C).

Inner caudal bristles as in the type form.

Tergal plates as in the type form except that the sixth and seventh segments show a marked tendency to the development of very small paired accessory tergal plates or plaques.

PUPA (fig. 6, B, D). *Bristle A* on segment VIII with 6 or 7 branches. *Apical hair of paddle* longer than in type form of *garnhami* and with distinct hooked shape.

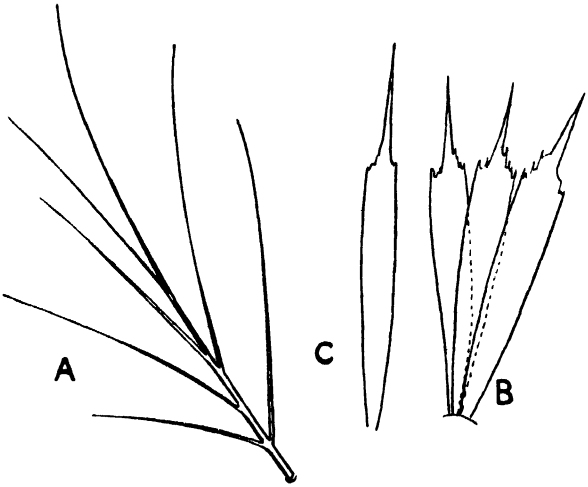

FIG. 9. *A. garnhami* var. *walshi*. A.—hair representing thoracic palmate hair. B.—leaflet from typical abdominal palmate hair. C.—leaflet with unusually long filament from typical abdominal palmate hair. Original.

DISTRIBUTION. Only known up to the present from Drakensberg, 3,000 to 4,000 ft., near Tzaneen, Transvaal, and Tzaneen itself, 2,380 ft., 15 miles from the mountains, where a few specimens have been collected. Cotype ♂♂ and ♀♀ from Magoeba's Kloof, Tzaneen, North Transvaal, 17.9.1932, collected by one of the authors (B. De M.).

ACKNOWLEDGMENTS

We are greatly indebted to Dr. F. W. Edwards for the loan of specimens in the collections of the British Museum, and also to Mr. C. B. Symes, of Kenya, and to Mr. G. H. E. Hopkins and Mr. E. G. Gibbins, of Uganda, for material of adults and larvae of East African *garnhami* and *demeilloni*.

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THE DISTRIBUTION OF BLACKWATER FEVER IN CENTRAL AMERICA, SOUTH AMERICA AND THE WEST INDIES*

BY

J. W. W. STEPHENS

(*Received for publication*, 1 *May*, 1933)

TWO MAPS

CENTRAL AMERICA.

Locality and Date	Cases	Authority
General	'In Central America the fever occurs upon the Isthmus of Panama, in Honduras, Nicaragua, Costa Rica, and along the entire coast-line' (p. 392).	Craig, C. F. (1909).
	'It is prevalent in Central America, particularly in Honduras, Nicaragua, and Costa Rica .... 'Numerous cases have been reported from Panama' (p. 34).	Deaderick, W. H. (1909).
		Deaderick, W. H., and Thompson, L. (1916). (p. 226).

COSTA RICA.

Locality and Date	Cases	Authority
Punta-Arenas ... 1864	'La fièvre rémittente bilieuse semble y avoir été observée assez souvent et y avoir été parfois confondue avec la fièvre jaune' (p. 374).	Le Roy de Méricourt, A. (1864).
Port Limon	'On the coast here we have many cases, although they bear a very small proportion to the enormous number of intermittent and remittent fevers' (p. 259). 3 cases reported	Steggall, S. L. J. (1899).
Costa Rica	Cases Deaths Year 15 5 (1900-1901) 11 ... (1913) 50 ... (1914)	United Fruit Company Medical Department Annual Reports (1912-1931).

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*STEPHENS, J. W. W. (1927). The distribution of blackwater fever in Europe. *Ann. Trop. Med. & Parasitol.*, **21**, 467.
——— (1928). The distribution of blackwater fever in South-West Asia. *loc. cit.*, **22**, 53.
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——— (1929). The distribution of blackwater fever in North America. *loc. cit.*, **23**, 451.

COSTA RICA—*continued*

Locality and Date	Cases			Authority
Costa Rica—contd.	Cases	Deaths	Year	United Fruit Company Medical Department Annual Reports (1912-1931).
	7	3	(1915)	
	3	2	(1916)	
	7	6	(1917)	
	21	5	(1918)	
	20	5	(1919)	
	17	4	(1920)	
	12	1	(1921)	
	15	9	(1922)	
	15	5	(1923)	
	18	2	(1924)	
	17	3	(1925)	
	32	7	(1926)	
	39	11	(1927)	
	22	3	(1928)	
	21	6	(1929)	
	35*	10	(1930)	
	28*	7	(1931)	

* In the Compania Bananera division, on the Atlantic Coast of Costa Rica.

GUATEMALA.

Locality and Date	Cases			Authority
	Cases	Deaths	Year	
	8	0	(1912)	United Fruit Company Medical Department Annual Reports (1914-1931)
	4	0	(1913)	
	13	2	(1914)	
	10	2	(1915)	
	7	2	(1916)	
	1	...	(1917)	
	4	1	(1918)	
	2	1	(1919)	
	1	...	(1921)	
	8	4	(1922)	
	5	2	(1923)	
	9	1	(1924)	
	7	2	(1925)	
	22	3	(1926)	
	13	7	(1927)	
	7	4	(1928)	
	8	3	(1929)	
	15	2	(1930)	
	21	6	(1931)	

HONDURAS.

Locality and Date	Cases			Authority
Atlantic Coast: *Ceiba*	'A la "Ceiba" j'ai eu maintes occasions d'observer la fièvre bilieuse hémoglobinurique qui dans cet endroit est connue généralement sous le nom de "Fiebre de Aquas Negras".'			Muñoz, F. R. (1920)
	Cases		Year	
	5		(1918-)	
General	'in Honduras noch haufiger ist'			Mense, C. (1924)
Atlantic Coast:	Cases	Deaths	Year	United Fruit Company Medical Department Annual Reports (1914-1931)
Tela	13	0	(1914)	
	20	4	(1915)	
Tela and Truxillo:	13	1	(1917)	
Tela	7	...	(1918)	
Truxillo	...	...	(1918)	
Tela	9	3	(1919)	
Truxillo	...	...	(1919)	
Tela	11	3	(1920)	
Truxillo	1	...	(1920)	
Tela	11	4	(1921)	
Truxillo	1	...	(1921)	
Tela	3	2	(1922)	
Truxillo	16	5	(1922)	
Tela	6	2	(1923)	
Truxillo	25	2	(1923)	
Tela	13	3	(1924)	
Truxillo	17	7	(1924)	
Tela	11	2	(1925)	
Truxillo	13	5	(1925)	
Tela	7	0	(1926)	
Truxillo	12	4	(1926)	
Tela	6	0	(1927)	
Truxillo	23	7	(1927)	
Tela	4	1	(1928)	
Truxillo	30	6	(1928)	
Tela	7	3	(1929)	
Truxillo	25	1	(1929)	
Tela	9	3	(1930)	
Truxillo	50	19	(1930)	
Tela	6	3	(1931)	
Truxillo	39	8	(1931)	
Tela	16*	3	(1931)	

* Cortes Development Company, Atlantic Coast

HONDURAS, BRITISH.

Locality and Date	Cases			Authority
Honduras (British)	Records 3 cases.			Browne, O. (1899).
	Cases	Deaths	Year	British Honduras Official Medical Reports.
	Not recorded		(1896-1922)	
	1	...	(1923)	
	1	...	(1925)	
	2	...	(1926)	
	11	1	(1930)	

MEXICO.

Locality and Date	Cases	Authority
Vera Cruz	'Im September des Jahres 1881 .... welcher seit 3 Tagen an Wechselfiebers ... erkrankt war. Der Vater hatte den Kranken schon mit Chinin behandelt und mich nur consultirt weil ihn die auffallend schwarze Farbe des Urins beunruhigte ... Der sehr reichlich gelassene Urin war von tief schwarzrother Farbe und reagirte deutlich sauer ...' 'Die in meinem Hause fortgeführte Untersuchung erwies sowohl durch Anwendung der Heller'schen Blutprobe, als durch die Beobachtung mit einem Zeiss'schen Mikrospektroskop die Anwesenheit von Methamoglobin im Urin' (p. 517).	Heinemann, C. (1885 and 1886).
Tampico	'Recent articles condemning quinine in this condition have induced me to report the following cases (2) selected from those treated by me while in charge of the Hospital Americano at Tampico.' 2 (1895) "These cases present an atypical course," the author states, (and I think the diagnosis is doubtful in both).	Livingston, W. R. (1897).

NICARAGUA.

Locality and Date	Cases	Authority
San Juan del Sur 1850	'Fluit dagegen hat seit 1850 in San Juan del Sur zahlreiche Falle bei Fremden, einige bei Eingeborenen gesehen, die meisten wahrend der feuchten euchten Jahrezeit' (p. 228).	Mense, C. (1899).
Managua	'E. Rothschuh schreibt aus Managua in Nicaragua, das er etwa 20 Falle behandelt habe, vorweigend bei Weissen, aber auch bei einzelnen Ladinos d. h. Mischlingen und reinen Indianern' (p. 228).	Mense, C. (1899).

PANAMA.

Locality and Date	Cases	Authority
General 1864	'*Les fièvres intermittentes* dominent la pathologie' (p. 285). '*La fièvre rémittente bileuse* y est fréquente' (p. 286).	Le Roy de Méricourt, A. (1864).

PANAMA—*continued.*

Locality and Date	Cases	Deaths	Year	Authority
Ancon, Canal Zone ...	14 (10 Nov., 1905—10 May, 1906). Nine of the cases occurred in Americans, two in Spaniards, one in an Italian, and one in a Spanish-American.			Brem, W. V. (1906).
Panama	Observer Gorgas; Place Panama; Time 8 months; Cases 20 (p. 393)			Craig, C. F. (1909).
Republic of Panama ...	11 (1918) All of these patients were negro or a mixture of negro and Spanish.			Ball, E. (1923).
Atlantic Coast :	Cases	Deaths	Year	
Bocas del Toro ...	...	1	(1900)	United Fruit Company Medical Department Annual Reports (1900-1931)
	...	1	(1902)	
	...	1	(1904)	
	...	1	(1906)	
	...	1	(1907)	
	...	6	(1908-09)	
	...	3	(1909-10)	
	...	2	(1910-11)	
	7	3	(1913)	
	12	1	(1914)	
	9	...	(1915)	
	6	1	(1916)	
	8	...	(1917)	
	4	1	(1918)	
	8	1	(1919)	
	7	1	(1920)	
	10	1	(1921)	
	5	2	(1922)	
	11	4	(1923)	
	9	3	(1924)	
	9	3	(1925)	
	4	2	(1926)	
	4	...	(1927)	
	7	1	(1928)	
	6	...	(1929)	
	13	1	(1930)	
	3	1	(1931)	
Pacific Coast :				
Chirigui	16	3	(1929)	United Fruit Company Medical Department Annual Reports (1929-1931).
	27	6	(1930)	
	13	3	(1931)	
Panama Canal Zone ...	...	17	(1906)	Annual Reports of the Department of Health (Sanitation) of the Panama Canal (1906-1930).
	...	20	(1907)	
	...	27	(1908)	
	...	15	(1909)	
	...	18	(1910)	
	...	42	(1911)	
	...	14	(1912)	
	...	13	(1913)	
	7	3	(1914)	
	4	4	(1915)	

PANAMA—*continued.*

Locality and Date	Cases	Authority
Panama Canal Zone—continued	1 ... (1918)	
	2 ... (1919)	
	1 1 (1920)	
	2 3 (1921)	
	... 1 (1923)	
	... 3 (1924)	
	2 1 (1925)	
	1 1 (1926)	
	2 2 (1927)	
	... 1 (1928)	
	1 3 (1929)	
	... 2 (1930)	

SOUTH AMERICA.

Locality and Date	Cases	Authority
General : *Argentina* ... ... *Brazil* ... .. *Guiana* ... ... *Venezuela*	'In South America the disease occurs in Venezuela, Guiana, the Argentine Republic and Brazil' (p. 392).	Craig, C. F. (1909).
General : *Brazil* ... .. *Guiana* ... .. *Venezuela*	Deaderick and Thompson state that hemogobinuric fever prevails more notably on the north and east coasts, in Venezuela, Guiana, and Brazil, at least as far South as Rio de Janeiro (p. 226).	Deaderick, W. H. (1909). p. 35 Deaderick, W. H., and Thompson, L. (1916).

ARGENTINE.

Locality and Date	Cases	Authority
General	'The disease occurs in . . . the Argentine Republic' (p. 392).	Craig, C. F. (1909).
General	'*L'emoglobinuria*, in forma grave ed anche mortale, non è rara.'	Alfaro, G. A. (1927).
North Argentine ...	'La *hemoglobinuria* en forma grave y aun mortal no es rara' (p. 174).	Alfaro, G. A. (1926).
Salta	'La *fiebre hemoglobinúrica* se observa con extraordinaria rareza habiéndosela visto sólo en niños' (p. 367).	Aranda, C. A. (1926).

ARGENTINE—*continued.*

Locality and Date	Cases	Authority
Tucumán	'Las hematurias observadas por varios colegas, no las catalogo como producidas por el paludismo por carecer de datos precisos' (p. 350).	Cossio, R. (1926).
Province of Buenos Aires ...	1 case (1 Feb., 1927) Malaria in Italy 1924. Left Italy October, 1926. Castex recorded a case in 1916 and one in 1919.	Castex, M. R. González, H., Poletti, R. A. (1928).
Jujuy	'Primer caso auctóctono de fiebre hemoglobinúrica observado en Jujuy' (p. 182). 'De frecuentes encuestas . . . he podido recoger la impresión de que se produjeron con anterioridad y en diferente épocas casos de fiebre hemoglobinúrica en la región y sobre todo en la zona fronteriza con Tucumán de la provincia de Salta. Así el doctor de Cores, de Rosario de la Frontera (Salta) tuvo oportunidad de atender, haec tres años dos niñas, de una misma familia, una de las cuales . . . emisión de orinas rojovinosas . . . vómitos incoercibles, de aspecto porraceo . . . La otra hermana que sólo presentó una emisión urinaria coloreada e ictericia.'	Mazza, S. (1932).

BRAZIL.

Locality and Date	Cases	Authority
Rio de Janeiro ...	'Auch die Professoren Moncorvo Vater und Sohn haben in Brasilien bei dem reichen Material der Kliniken von Rio de Janeiro hämoglobinurisches Fieber haufig zu behandeln gehabt, ersterer in etwa 100, letzterer in 10 Fallen (2 Neger).'	Mense, C. (1899)
General	'In America it has been encountered in French Guiana, Venezuela, Cuba and elsewhere, but it is apparently unknown in Brazil' (p. 485).	Marchiafava and Bignan. (1900)
Rio de Janeiro ...	'In S. America hemoglobinuric fever prevails . . . at least as far South as Rio.'	Deaderick, W. H. (1909), p. 35. Deaderick, W. H., and Thompson, L. (1916), p. 226.
Amazonas	35 cases 'Blackwater is frequent in the province of Amazonas.'	de Matta, A. A. (1912).

BRAZIL—*continued.*

<table>
<tr><th>Locality and Date</th><th>Cases</th><th>Authority</th></tr>
<tr><td><i>Madeira-Mamore Railway Co., Porto Velbo</i> ...</td><td>Racial incidence of blackwater fever.
<table>
<tr><th>Nationality</th><th>Hospital admissions*</th><th>Black-water cases</th><th>Rate per 1,000 admissions</th></tr>
<tr><td>West Indians ...</td><td>4,537</td><td>5</td><td>1·02</td></tr>
<tr><td>Brazilians ...</td><td>5,751</td><td>52</td><td>9·04</td></tr>
<tr><td>North Americans plus Europeans other than Spaniards ...</td><td>8,638</td><td>145</td><td>16·78</td></tr>
<tr><td>N. Americans plus all Europeans</td><td>14,759</td><td>390</td><td>26·42</td></tr>
<tr><td>Spaniards ...</td><td>6,121</td><td>245</td><td>40·02</td></tr>
</table>
*For the period of 46 months: Jan 1, 1908-Nov. 27, 1912.</td><td>Lovelace, C. (1913).</td></tr>
</table>

COLOMBIA.

<table>
<tr><th>Locality and Date</th><th>Cases</th><th>Authority</th></tr>
<tr><td><i>General</i> ... ...</td><td>‘La fiebre hemoglobinúrica no parece exister en Colombia sino en los focos mas intensos de Paludismo Chocó, márgeres del Magdalene en determinados puntos, Santa Marta, reberas del Zulia etc’ (p. 129).</td><td>Marquez, L. C. (1915).</td></tr>
<tr><td><i>Bogota</i> ... ...</td><td>‘<i>Fiebre baemoglobinurica</i> (Black-water fever). This complaint is observed amongst those attacked by chronic malaria . . .’</td><td>Santamaria, J. M. (1913).</td></tr>
<tr><td><i>General</i> ... ...</td><td>‘Malaria findet sich in den feucht warmen Ebenen haufig; auch Schwarzwasserfieber kommt vor’ (p. 359).</td><td>Muhlens, P. (1920).</td></tr>
<tr><td></td><td>
<table>
<tr><th>Cases</th><th>Deaths</th><th>Year</th></tr>
<tr><td>6</td><td>3</td><td>(1916)</td></tr>
<tr><td>2</td><td>...</td><td>(1917)</td></tr>
<tr><td>1</td><td>...</td><td>(1919)</td></tr>
<tr><td>1</td><td>...</td><td>(1923)</td></tr>
<tr><td>2</td><td>1</td><td>(1924)</td></tr>
<tr><td>1</td><td>1</td><td>(1925)</td></tr>
<tr><td>2</td><td>1</td><td>(1926)</td></tr>
<tr><td>1</td><td>1</td><td>(1929)</td></tr>
<tr><td>3</td><td>1</td><td>(1930)</td></tr>
<tr><td>2</td><td>...</td><td>(1931)</td></tr>
</table></td><td>United Fruit Company Medical Department Annual Reports (1916-1931).</td></tr>
<tr><td><i>Barranca-Bermeja Santander</i> ... ...</td><td>‘20 Cases of blackwater fever from the interior of Colombia.’</td><td>Paterson, J. C. (1933).</td></tr>
</table>

ECUADOR.

Locality and Date	Cases	Authority
Guayaquil 1864	' D. *Fièvre remittente bilieuse.*—Elle est assez fréquente et assez grave pour avoir fait croire plusieurs fois à l'invasion de la fièvre jaune ' (p. 281).	Le Roy de Mericourt (1864).

GUIANA, BRITISH.

Locality and Date	Cases			Authority
	Cases	Deaths	Year	British Guiana Official Medical Reports.
	...	11	(1913)*	
	...	11	(1914)*	
	...	19	(1915)*	
	...	20	(1916)*	
	...	17	(1917)*	
	...	10	(1918)*	
	...	8	(1919)*	
	...	6	(1920)*	
	...	4	(1921)*	
	...	4	(1922)*	
	...	...	(1923)	
	...	...	(1924)	
	...	7	(1925)	
	...	8	(1926)	
	...	19	(1927)	
	...	6	(1928)	
	...	11	(1929)	
	...	12	(1930)	

*The data for these years kindly supplied by Surgeon-General Wise (Jamaica).

GUIANA, FRENCH.

Locality and Date	Cases			Authority
	C'est la seconde fois que la fièvre dite bilieuse inflammatoire de l'Amerique tropicale fait le sujet d'une étude méthodique et approfondie . . . M. Burot a retrouvé cette maladie à la Guyane.' (Review by A. Borius.)			Burot, P. (1881).
	Sur les statistiques . . . pour toute la Guyane.			Clarac (1902).
	Cases	Deaths	Year	
	27	4	(1897)	
	13	6	(1898)	
	10	5	(1899)	
Saint-Jean-du-Maroni	20	11	16 juin, 1887- 31 décembre 1899)	

GUIANA, FRENCH—*continued.*

Locality and Date	Cases	Authority
General	'On rencontre toutes les formes de l'intoxication palustre ; accès pernicieux, fièvres bilieuses hémoglobinuriques, typho-malariennes ; toutes ces formes sont également fréquentes et également graves ' (p. 375).	Clarac (1907).
General	' In Franzosich Guayana fand Theze es selten ' (p. 485).	Mense, C. (1924).

PARAGUAY.

Locality and Date	Cases	Authority
General	' . . . Paraguay. He (the reviewer) was there quite recently. *Leishmaniasis* is very common indeed in that country, and so are infections with *P. falciparum* ; and blackwater fever is not rare ' (p. 186).	James, W. M. (1931).

URUGUAY.

Locality and Date	Cases	Authority
	' L'Uruguay est le seul pays de l'Amérique du Sud où on n'a pas encore signalé le paludisme autochtone.'	Talice, R. V. (1931).

VENEZUELA.

Locality and Date	Cases	Authority
General	' In French Guiana, Venezuela, Cuba and elsewhere ' (p. 486).	Marchiafava and Bignami (1900).
	' Haemoglobinuric fever . . . in Venezuela ' (p. 226).	Deaderick and Thompson (1916).
	' Auch Schwarzwasserfieber est nicht unbekannt.'	Muhlens, P. (1920).
R. Orinoco	' Ist es etwas haufiger in den Niederungen des Orinokko und seiner Nebenflusse ' (p. 485).	Mense, C. (1924).
General	Numerous papers on the subject of blackwater fever from 1881 onwards have appeared in Venezuelan journals unavailable in this country.	Venezuelan Journals.

WEST INDIES.

Locality and Date	Cases	Authority
*Antilles**	'C'est particulièrement dans les foyers d'émanations palustres très-intenses de Fort-de-France et de la Pointe-à-Pitre, dans ce dernier surtout, qu'on la rencontre. Dans les localités . . . . comme Sainte-Pierre et la Basse Terre, elle est rare, et si elle apparaît, c'est qu'elle a été contracteé ailleurs . . . . c'est seulement dans les lieux marécageux, là ou règne la fièvre paludéenne grave qu'on l'observe.' (la fièvre bilieuse grave) (p. 316.) 'Telle est la fièvre que les medecins de la Pointe-à-Pitre, où elle est plus souvent observée qu'ailleurs ont nommée *fièvre bilieuse hématurique* , *fièvre jaune des acclimatés et des créoles*' (p. 318).	Dutroulau, A. F. (1868).
1828-1838	'Ce practicien (Lherminier) . . . . reconnait que la fièvre bilieuse, était plus grave autre fois qu' aujourd'hui : de 1828 à 1838 époque d'immunité pour la fièvre jaune, il l'a observée frequemment sur les créoles' (p. 318).	
Pointe-à-Pitre... ... 1853-1854	'La fièvre bilieuse hématurique des Antilles . . . . n'est pas une maladie fréquente ; elle est entièrement étrangère à certaines localités, . . . Les années 1853 et 1854 en on offert d'assez nombreux exemples à Pointe-à-Pître' (p. 321).	Dutroulau, A. F. (1868).
General	'In West Indies, blackwater fever occurs in Cuba, Martinique, Guadeloupe, Trinidad and some of the smaller islands belonging to this group' (p. 392).	Craig, C. F. (1909).
Guadeloupe, *Hayti*, *Martinique*	'It is found in the Greater Antilles, but appears to be rare in Hayti. In the Lesser Antilles it is more common on the islands of Guadeloupe and Martinique.	Deaderick, W. H. (1909), p. 34. Deaderick and Thompson (1916), p. 226.

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* The French Antilles (Les petites Antilles) comprise (1) Gaudeloupe and its dependencies, Les Saintes, Marie galante, la Désirade, St. Barthélémy, (2) St. Martin (part of), (3) Martinique.

ANTIGUA.

Locality and Date	Cases	Authority
Antigua	'The urine also in those aggravated cases (of bilious fevers) was always very acid, the secretion scanty and high coloured, sometimes even bloody' (p. 34). (It is doubtful, I think, whether these were cases of blackwater fever).	Freeland, J. (1891).

CUBA.

Locality and Date	Cases			Authority
Santiago da Cuba ...	'The malignant type seems to be the endemic form of malaria parasite in this region.' 'In three cases of malarial haematuria or rather haemoglobinuria, a few hyaline unpigmented bodies were found.'			Fabricius, F. W. (1899).
Santiago General Hospital.	6 (1898-99) (p. 190)			Smith, B. (1900).
Santiago	'I recollect very few cases of pernicious malaria or blackwater fever.'			Gorgas, W. C. (1906).
Cuba :—	Cases	Deaths	Year	United Fruit Company Medical Department Annual Reports (1914-1931).
Preston	2	...	(1914)	
Banes	4	...	(1915)	
Preston	3	1	(1915)	
Banes	3	1	(1916)	
Nipe Bay ...	7	2	(1917)	
Banes	7	3	(1917)	
Preston	1	...	(1918)	
Banes	3	1	(1918)	
Preston	...	...	...	
Banes	5	13	(1919)	
Preston	4	1	(1919)	
Banes	8	1	(1920)	
Preston	...	...	...	
Banes	22	2	(1921)	
Preston	3	1	(1921)	
Banes	2	...	(1922)	
Preston	...	1	(1922)	
Banes	6	1	(1923)	
Preston	2	2	(1923)	
Banes	4	2	(1924)	
Preston	1	...	(1924)	
Banes	42	10	(1925)	
Preston	1	1	(1925)	
Banes	75	13	(1926)	
Preston	12	2	(1926)	
Banes	38	9	(1927)	
Preston	16	4	(1927)	
Banes	7	1	(1928)	
Preston	5	1	(1928)	
Banes	9	3	(1929)	
Preston	4	2	(1929)	
Banes	1	...	(1930)	
Preston	2	1	(1930)	
Banes	...	*2	(1931)	

* Cuban Divisions = Banes and Preston.

GUADELOUPE.

Locality and Date	Cases	Authority
Guadeloupe 1848	'Maladie paludéenne ictérique. (Fièvre jaune ; typhus ictérode ; fièvre rémittente bilieuse ; fièvre rémittente perniciouse mélanurique).' 'Obs IX Mademoiselle A . . . . 17 ans.' '17 février, 1848. Fièvre ; 100 pulsations ; les yeux, le pourtour des lèvres ont une legère teinte jaune . . . . (Frictions avec sulfate de quinine 2.50 grammes ; à l'intérieur, sulfate de quinine 1.10 . . . .' '18 février . . . . les urines avaient prises une couleur noire.' '22 février . . . . vomissements noir . . . . La malade périt.' 'Obs X. Mademoiselle A . . . . 23 ans.' '12 mai . . . . frisson violent à minuit ; vomissements bilieux . . . . (sulfate de quinine 1.50 ; quinquina, 30 grammes).' '14 . . . . depuis le premier frisson les urines sont noires, la face d'un jaune intense.'	Duchassaing, A. (1850).
1847	'Obs. XI. L . . . . de race blanche 14 ans.' '17 et 18 decembre, 1847. Un faible accès de fièvre intermittente.' '19. Troisième accès plus fort. Il prend du sulfate de quinine qu'il vomit.' '20. A huit heures du matin, frisson violent, face d'un jaune intense . . . . Urines noires.' '21. Vomissements de matières noirâtres.' There is no evidence in this paper, if as the title implies it is dealing with blackwater fever, that Duchassaing attributed the black urines to quinine as stated by Corre (1892). (q.v.).	
Pointe-à-Pitre ... 1859	'Continué plus longtemps le traitement quinique peut constituer un danger, et on n'est pas éloigné à la Pointe-à-Pitre, d'attribuer à l'abus qu'on en faisait autrefois les accidents hémorrhagiques qui étaient bien plus fréquents alors qu' aujourd'hui.'	Dutroulau, A. F. (1859).
Pointe-à-Pitre ...	1 (1861)	Pellarin, A. (1862).
Islet of Cosson ...	'Six années de service en Afrique. Dix-sept mois de séjour à la Pointe-à-Pitre. Réside dépuis cinq mois, à l'îlet à Cosson.' 'Toutes les formes des fièvres et notamment la fièvre hématurique y sont endémiques.'	Pellarin, A. (1862).

GUADELOUPE—*continued.*

Locality and Date	Cases	Authority
Basse-Terre, *Grande-Terre,* *Pointe-à-Pitre.*	'F . . . . vient de passer deux ans en détachement à la Pointe-à-Pitre où il a eu nombreux accès de fièvre : arrivé à la Basse-Terre il ressent le jour même un malaise qui persiste deux jours ; un accès avec frisson intense se déclare pendant lequel le malade dit avoir rendu les urines noires. Le 25. Entré à l'hôpital le soir même, le malade presente les symptômes suivants : teinte ictèrique de la peau. . . . Les urines du soir sont couleur du vin de Malaga . . . . vomissements bilieux d'un vert porracé, une selle liquide de même nature. . . . Le 28. Au matin les vomissements deviennent plus fréquents. . . . Les urines sont rougeâtres ; deux selles de même couleur. . . . les vomissements sont incoercibles. Emission des urines difficile ; les urines sont plus rares, mais de même couleur. Le 30. Les vomissement cessent, les urines sont peu colorées . . . le délire survient, l'etat comateux lui succède et le mort arrive le 30 au soir.' 'Ces cas sont très rares à la Basse-Terre ; ils sont plus fréquents à la Pointe-à-Pitre et à la Grande-Terre.'	Brassac, P. J. M. (1863).
Saint-Jules Hospital ...	1 (1862) 'La fièvre bilieuse hématurique improprement appelée fièvre jaune des créoles. . . .' '. . . . la fièvre bilieuse hématurque qui serait mieux nommée néphrorrhagique. 'Sur sept cas mortels. . . . L'intelligence est restée intacte jusqu'au moment de la mort.'	Pellarin, A. (1865). **3**
Pointe-à-Pitre Hospital	1 (1864)	Pellarin, A. (1865). **4**
Basse-Terre	'M. C. de L. . . . âgé de 36 ans, né à la Basse-Terre (Guadeloupe). Entré à l'hôpital le 27 Octobre, 1870. . . mort le 29 du même mois.'	Manceaux, T. L. (1872).
Pointe-à-Pitre... ...	'M. Raimond relate cinq cas observés à la Pointe-à-Pitre, dans les mois les plus froids : décembre, janvier, février et mars (de la fièvre bilieuse hématurique) (p. 79).	Raimond (1874), quoted by Brejon, F. (1881).
Guadeloupe	'C'est la maladie que le peuple nomme à la Guadeloupe, ou je l'ai observée, *fièvre à urines noires*, fièvre jaune des créoles, et que le plupart des médecins nomment aujourd'hui fièvre bilieuse hématurique' (p. 85). Cases Year 1 (1860) 4 (1861) 5 (1862) ? (1863) 3 (1864) pp. 184-311.	Pellarin, A. (1876).

GUADELOUPE—*continued.*

Locality and Date	Cases	Authority
Guadoloupe—continued	'À la Guadeloupe, la maladie est commune dans les endroits ravinès, dans les coulées que balaient les fortes brises; elle sévit durant les mois les plus frais (décembre-mars) et apparâit souvent à l'occasion d'un changement de résidence, d'une émigration sur le littoral; chez quelques sujets la susceptibilité relative aux écarts de la température relèverait d'une idiosyncracie héréditaire, du moins le Dr. Lherminier nous a dit avoir constaté une sorte de prédisposition aux accès jaunes (autre nom de la fièvre hémoglobinurique aux Antilles) dans certaines familles' (p. 376).	Corre, A. (1887).
	'La méthémoglobinurie quinique.' 'La premiére mention qui ait été faite d'urines noires provoquées par l'administration des sels de quinine, appartient à un medecin de la Gaudeloupe, Duchassaing' (p. 320).	Corre, A. (1892).
	'Ici, la bilieuse hémoglobinurique est plus souvent observée. "La morbidité par paludisme est par contre assez élevée, surtout à la Guadeloupe, la grande Terre est surtout insalubre, la Pointe-à-Pitre est bâtie sur un ancien marais; plus de la moitié des hommes de troupe est atteinte. Ici, la bilieuse hemogloburinurique est plus souvent observée." (As compared with Martinique)' (p. 567).	Brault, J (1907)
	'. . . . La forme hémoglobinurique inconnue à la Martinique est très fréquente à la Guadeloupe. Dans les régions nord et nord-est (Morne-à-l'Eau, Canal, Port-Louis, la Pointe-à-Pitre, sur la Grande-Terre; Sainte Rose, Deshaies, Pointe Noire, Bouillante, Goyaves, à la Gaudeloupe proprement dite), la bilieuse hémoglobinurique se manifeste avec une fréquence telle que plusieurs considèrent les sels de quinine comme jouant un rôle important dans la pathologie de cette manifestation. La vérité est que les Antillais de ces régions présentent une véritable idiosyncrasie et font de la bilieuse hémoglobinurique ou de l'hémoglobinurie quinique avec une singulière facilité; chez les uns, c'est l'intoxication paludéenne grave qui est en cause; chez les autres c'est l'intoxication quinique qui intervient sans qu'il soit permis de considérer les deux manifestations comme identiques, et cela pas plus à la Guadeloupe qu'ailleurs. Dans cette colonie comme ailleurs, le paludisme grave est facteur de la bilieuse hémoglobinurique, qui ne se rencontre que dans les régions ou l'endémie sévit avec intensité' (p. 343).	Clarac (1907).

HAITI.

Locality and Date	Cases	Authority
Port Goâve	Mr. G. D., pharmacien, établi depuis cinq mois seulement à Port Goâve (Haïti), ville où la malaria est à l'état endémique a été atteint de fièvre avec frissons.' 'Le premier jour il prend 1 gr. 50 centigrammes de sulfate de quinine en 12 heures . . .' 'Le 2ème jour le malade avale deux grammes toujours en 12 heures.' 'Les urines deviennent noires. Température 39°.' 'Le 3ème jour suppression de la quinine et les urines deviennent normales le soir.' 'Le 4ème jour la fièvre avait complètement disparu avec tous les symptomes.'	Sylvain, E. (1899).
Santo Domingo La Romana.	'Three of these were typical cases of blackwater fever.'	Otis, E. F. (1915).
Haiti	'6 cases among U.S. marines, 1 case in a French priest who had lived in Haiti 13 years.'	Torrance, R. A., and Bowman, F. H. (1917).
	'During this time (two and one-half years), 626 cases of malaria and 13 cases of blackwater fever have come under our observation. . . . In the early days of the occupation (1915-16) records show that troops serving in northern Haiti in the district around Fort Liberty and Ouanaminthe, had to be replaced frequently because of the prevalence of malignant malaria and blackwater fever. . . .' 'Of the 13 cases of blackwater fever which we have seen, seven cases came from Biziton and 4 from Mirebalais' (pp. 413-414).	Robertson, G. E., and Moore, W. (1923).
	'Blackwater fever, strange to say, is not frequently seen in its true form, although it is not at all unusual to see an apparently normal convalescent with hemoglobinuria. Where typical blackwater fever is present, it is almost invariably fatal' (p. 422).	Storch, R. B. (1923).
	8 cases in 30 years. All occurred amongst adult Haitians except one, who was a young Syrian' (p. 161).	Hudicourt, L. (1927).

JAMAICA.

Locality and Date	Cases	Authority
1859	‘Mr. R. T., planter, aged 37 . . . . was attacked with intermittent fever on Saturday, the 13th of August, 1859, and until Monday, the 15th, when I saw him, the fever had continued with very little intermission.’ ‘. . . . I found the urine to be of a dark porter colour, with a deposit of grumous sediment.’ ‘. . . . the evacuations were liquid, black, and very offensive . . . . the conjunctivae were deeply tinged with bile, and the upper part of the body was of a deep lemon colour . . .’ ‘16th . . . . The urine is still of a deep port-wine colour and very scanty; has vomited a good deal of green bilious matter.’ ‘18th . . . . *no secretion of urine.*’ ‘18th. Urine, which, up to this morning had been entirely suppressed for forty-eight hours, was passed in very small quantity.’ ‘20th . . . . secretion of urine has been again arrested.’ ‘24th-26th. The fever had evidently assumed a typhoid-remittent type . . . almost constant hiccough Urine . . . . totally suppressed for the last two days.’ ‘27th. I passed a catheter into the bladder, and drew off some turbid urine. But he died soon after. . . .’	Croskery, H. (1860).
Jamaica	Cases Deaths Year 1 ... (1926) 1 1 (1927)	United Fruit Company Medical Department Annual Reports, (1926-1931).
	4 ... (1911-1912) 8 1 (1913-1914) 2 1 (1914-1915) 5 2 (1915-1916) 8 1 (1916-1917) 1 ... (1919-1920) 1 ... (1920) 5 ... (1921) 5 2 (1924)	Jamaica Official Medical Reports.
General	1927 ‘Cases of blackwater fever occur, but as these are mainly in the private practice of medical men, no records are available’ (p. 20)	Jamaica Annual Report of the island Medical Department (1928).

MARTINIQUE.

Locality and Date	Cases	Authority
Saint-Pierre	Fièvre bilieuse grave. 'Cette fièvre. . . . tantôt *hémorrhagique* tantôt *ictérique*, etc., mais distincte cependant de la fièvre jaune, cette fièvre est rare à Saint-Pierre, ou du moins l'appareil symptomatique qu'on lui attribue y présente rarement le même developpement que dans les lieux où elle parait être endémique' (p. 36). 'On peut dire que l'hématurie, telle qu'elle est décrite pour l'île Maurice ou la Réunion, n'existe pas à la Martinique; je n'en ai pas vu un seul cas' (p. 273).	Rufz de Lavison (1869).
Fort-de-France Hospital	(Fièvre bilieuse hématurique). 'M. l'Abbé C. . . . âgé de 42 ans, d'une constitution ruinée par un très-long séjour dans les colonies, entre à l'ôpital le 4 Octobre et meurt le 19 du même mois.'	Manceaux, T. L. (1872).
	'La Bilieuse hémoglobinurique est rare (as compared with the prevalence in Guadeloupe).'	Brault, J. (1907).

ST. LUCIA.

Locality and Date	Cases	Authority
St. Lucia *1781*	Of the History of the Remittent. 'After the first remission . . . . all the preceding symptoms increased, with the addition of a foul tongue, a yellowness of the eyes, and in some cases, an universal tinge, delirium, urine in small quantities and very highly coloured, imparting an offensive smell, often a difficulty in voiding it, which sometimes came to a perfect stoppage. . . .' (p. 59). 'A comatose disposition, remarkable dejection, coldness of the skin, partial cold sweats, hiccup, involuntary stools, *subsultus tendinum*, loss of speech, etc., were certain signs of danger' (p. 60).	Rollo, J. (1781).
1831	Case LVI. *Intermittent; blood fluid.*— 'G.B., Esq., a planter . . . . . has suffered several attacks of intermittent . . . . June 17, 1831, ... great restlessness; tension of the abdomen; pain or (on) pressure over the epigastrium; considerable thirst; vomiting of everything taken into the stomach; tongue clean and moist; pulse 120, and weak; conjunctivae yellow; countenance anxious, fear of death.'	Evans, W. J. (1837).

St. Lucia—*continued.*

Locality and Date	Cases	Authority
St. Lucia—continued 1831	'18th, morning. Countenance very anxious; the whole body of a deep yellow colour; patches of cutaneous ecchymosis, here and there of a purple colour; . . . . has passed a quantity of urine of a black or port-wine colour, thick and turbid; this colour depends upon a dark-coloured substance diffused through it, and which when allowed to repose, falls to the bottom of the tumbler in the form of a dark precipitate, resembling black snuff; the super-jacent urine becoming clear and of a dark-red colour.' '26th. Patient is well' (p. 139).	
1830	Case LXIX. *Yellow suffusion, bloody urine, etc.* 'He has suffered two or three times from intermittents.... December 12th, 1830.... the conjunctivae and the surface of the body of a greenish yellow colour; patches of extravasation on the chest, but not extensive; incessant vomiting of green fluid . . . . difficulty and pain in micturition; the urine received in a tumbler is thick, turbid, of a dark colour, and deposits a quantity of grumous blood; . . . .' '. . . . the morning of the 13th . . . . passed his urine which no longer contained blood, but which threw down a lateritious sediment.' '14th . . . . convalescent' (pp. 167-167). *State of the Urine.* '. . . . It often contains blood, which falls to the bottom of the chamber-pot, in the form of a black grumous sediment; now and then it is clear, and of the colour of port-wine. Under every circumstance it is acid, and has a strong action on litmus paper' (p. 245). 'Several species of a spurious cinchona are found in many of the West India islands. That found in St. Lucia has been described under the names of quinine or Ecorce de Ste. Lucie; cinchona floribunda; cinchona montana; trachelium arborescens et fluviatile, etc., etc' (p. 309).	Evans, W. J. (1837).
St. Lucia	Cases Deaths Year No records (1920) " (1921) " (1922) ... ... (1923) ... ... (1924) ... ... (1925) 2 2 (1926) ... ... (1927) ... ... (1928) ... ... (1929)	St. Lucia Official Medical Reports.

ST. VINCENT.

Locality and Date	Cases		Authority
St. Vincent	Cases Not recorded	Year (1920-1930)	St. Vincent Official Medical Reports.

TRINIDAD AND TOBAGO.

Locality and Date	Cases					Authority
Trinidad	'Blackwater fever is so extremely rare in this Colony. . . . In fact it was believed not to exist in Trinidad. . . no cases were reported. During the last eighteen years' practice I have heard of only three cases, and the case under review. . . . all these cases occurred recently (two this year and two in 1898-9) and in different parts of the Island.'					Bennett ,R. C. (1900).
Trinidad and Tobago ...	Trinidad		Tobago			Trinidad and Tobago Official Medical Reports.
	Cases	Deaths	Cases	Deaths	Year	
	2	...	...	...	(1914-5)	
	...	...	...	...	(1905-6)	
	4	2	...	...	(1906-7)	
	0	4	...	...	(1907-8)	
	2	1	...	...	(1908-9)	
	...	3	...	...	(1909-10)	
	1	1	...	...	(1910-11)	
	1	1	...	...	(1911-12)	
	1	...	...	...	(1912-13)	
	1	1	...	...	(1913-14)	
	1	2	...	...	(1914-15)	
	...	1	...	...	(1915-16)	
	...	...	...	1	(1917)	
	...	1	...	1	(1918)	
	...	...	...	1	(1919)	
	...	1	...	...	(1920)	
	...	1	...	...	(1921)	
	...	...	...	...	(1922)	
	...	...	...	...	(1923)	
	...	5	...	...	(1924)	
	...	...	...	...	(1925)	
	...	...	...	...	(1926)	
	...	3	...	...	(1927)	
	...	1	...	...	(1928)	
	...	...	...	...	(1929)	
	20	4	...	...	(1930)	
	2	0	...	...	(1931)	

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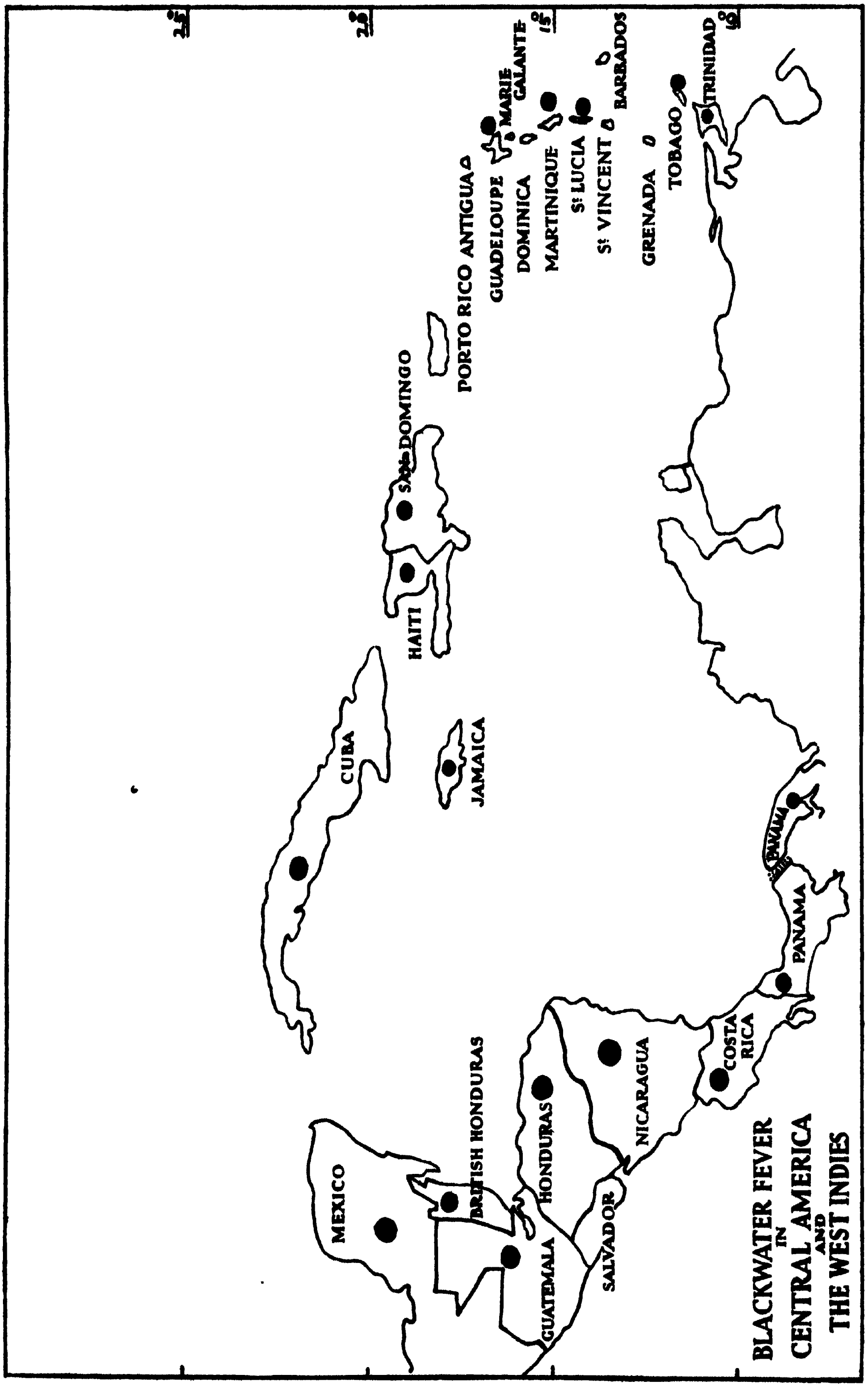
BLACKWATER FEVER
IN
CENTRAL AMERICA
AND
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GUATEMALA
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SALVADOR
NICARAGUA
COSTA RICA
PANAMA
PANAMA
CUBA
JAMAICA
HAITI
SAN DOMINGO
PORTO RICO
ANTIGUA
GUADELOUPE
MARIE GALANTE
DOMINICA
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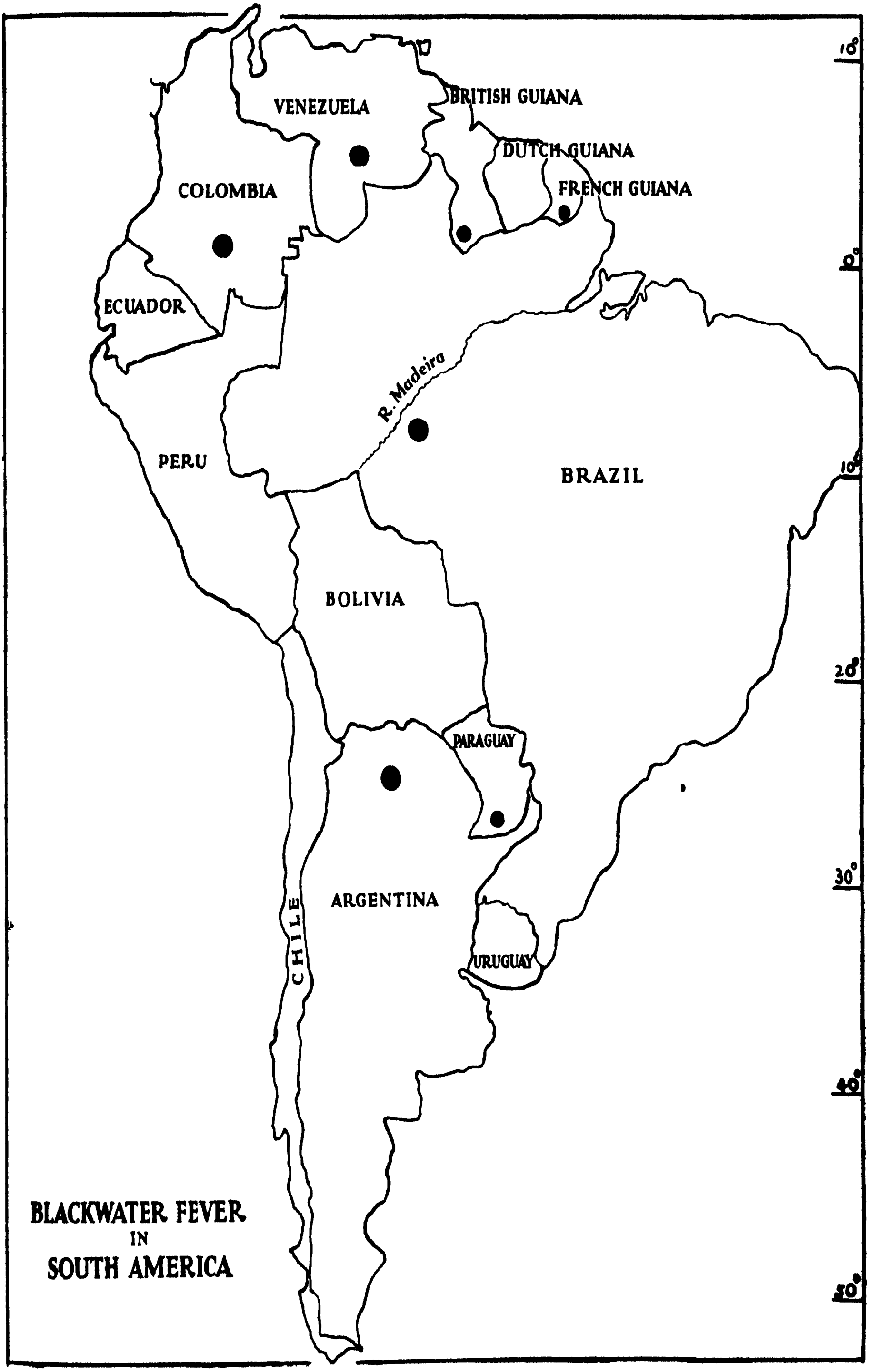

BLACKWATER FEVER IN SOUTH AMERICA

A RECORD OF AN INVESTIGATION INTO THE ACTION OF SERA ON THE TRYPANOSOMES PATHOGENIC TO MAN

BY

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In May, 1930, I was seconded for fifteen months to the Human Trypanosomiasis Institute, Entebbe, Uganda, to pursue a line of investigation into the action of human serum, *in vitro*, on the trypanosomes pathogenic to man. Unfortunately, from my point of view, *T. rhodesiense* has been found but recently and in odd cases only in the West Nile district of Uganda, and fresh strains of this parasite were unobtainable locally; there were, however, a number of strains of *T. gambiense* and *T. rhodesiense* in laboratory animals at Entebbe, and some initial work was performed with these to find out whether the technique for the maintenance of the parasites *in vitro* at 37° C. already described (Yorke, Adams and Murgatroyd, 1929) was satisfactory under the conditions obtaining there. The stock of laboratory animals consisted of monkeys, rabbits and guinea-pigs, whereas in the original work mice alone had been used as the source of the parasites. Guinea-pigs, although suffering from several disadvantages, more especially the slowness of development of an infection in them, were used for many experiments, and a technique was evolved whereby, even though the number of parasites in the peripheral blood was small, a sufficient quantity could be obtained by heart puncture and the centrifugation of several cubic centimetres of citrated blood in Ringer-glucose solution to enable experiments to be performed. The removal of the serum and red cells by centrifugation was followed by a single washing of the parasites in further Ringer-glucose solution, and the parasites were consequently obtained relatively free from the accompanying serum. Several attempts were made to wash the parasites in three or four changes of Ringer-glucose to free them yet more from serum, but owing to the inadequacy of the available centrifuge the period of time necessary for this treatment militated very considerably against its general usage ; a further deterrent to its routine adoption was the fact that the parasites were found to die relatively rapidly even at room temperature (25° C.), unless a reasonable proportion of serum was present in the suspending fluid.

Examination of a large number of sera as media showed that the deactivated sera of rabbits, sheep, goats, guinea-pigs and monkeys (*Cercopithecus* sp.) were suitable for the maintenance of the trypanosomes at 37° C. for periods up to 48 hours. Rabbit serum was at first used as the usual medium, and this was later replaced by the serum of a sheep—sheep 27—which was used consistently after it had been demonstrated that this particular serum possessed no antagonistic action to the trypanocidal body in normal human serum. The use of the serum of a single such animal has many practical advantages over that of sera from a variety of small animals such as rabbits or guinea-pigs, and the ease with which large amounts of serum could be obtained in a sterile manner at frequent intervals and with a minimum of preparation from such an animal greatly facilitated work under the existing conditions.

A systematic study of the reactions to normal human serum *in vitro* of the existing laboratory strains was undertaken, after the preliminary work on technique was completed, in order to determine whether any difference existed between the sera of Europeans and of natives. It was found that the serum of a normal healthy native was trypanocidal to the same degree as that of a healthy normal European, and, further, that when a number of healthy native sera were used in a single experiment consistently similar results were obtained in regard to the trypanocidal titres of the sera. The majority of the native bloods were found to contain various larval filariae, but infection with these helminths did not have any effect on the trypanocidal titre of the serum *in vitro.*

T. GAMBIENSE

Four strains of *T. gambiense* from Uganda were examined in regard to their resistance or susceptibility to the action of normal human serum, and in each case the trypanosomes were found to be completely resistant to its action *in vitro* for 24 hours at 37° C. over a period of twelve months' maintenance in laboratory animals. In Table I are set forth summaries of some results obtained with two of these strains, " Yembuga " and " Itaya," together with some details of the histories of their origin and maintenance. They were examined at more frequent intervals than is shown in the Table, but for the sake of brevity details of all the examinations are not given. From an inspection of Table I it is apparent that transmission neither by means of fly passage nor by syringe passage in a variety of laboratory animals affected their resistance to the action of normal human serum. The two other strains were studied over the same period, and similar results were obtained in each case. These results tend to confirm the observations of Yorke, Adams and Murgatroyd (1930) that human serum fastness is a characteristic of *T. gambiense* and is maintained for a considerable period of time through repeated passages in laboratory animals.

The reactions of these same strains to the sera of animals experimentally infected and of a man naturally infected with *T. gambiense* were also investigated; in addition, they were tested against the

TABLE I

Showing the results of examination over a period of two strains of *T. gambiense*, one maintained by several fly passages and the other entirely by syringe inoculation, for their reaction to normal human serum.

Strain	Origin of strain	Maintenance of strain	Animals examined	Dates of examinations	Reaction to human serum
Yembuga	Man; Uganda, 31.1.29	By fly from man into a sheep; then by fly through 3 monkeys; then by fly into a guinea-pig; and since by syringe in guinea-pigs.	Guinea-pig 11A, 1st Guinea-pig (by fly from 3rd monkey)	28.8.30	Resistant to pure human serum for 24 hours at 37° C.
			Guinea-pig 11B, 2nd Guinea-pig	17.9.30	"
			" 11B, 2nd "	3.10.30	"
			" 11C, 3rd "	16.11.30	"
			" 11E, 4th "	31.12.30	"
			" 77B, 6th "	21.7.31	Still completely resistant to pure human serum.
Itaya ...	Man; Uganda, 28.11.29	By syringe inoculation into a guinea-pig, and since maintained by syringe passage in 7 guinea-pigs and finally in mice.	Guinea-pig 13A, 3rd Guinea-pig	10.8.30	Resistant to pure human serum for 24 hours at 37° C.
			" 13A, 3rd "	15.9.30	"
			" 56B, 4th "	14.11.30	"
			" 13B, 5th "	31.12.30	"
			" 62A, 7th "	1.7.31	"
			Mouse 157A, 1st Mouse	29.7.31	"
			" 173A, 2nd "	5.8.31	Still completely resistant to pure human serum.

sera of such naturally resistant animals as the baboon. Results of such experiments with one of the strains are set forth in Table II, from which it will be observed that while a definite complete resistance is present against pure normal human serum, such is not the case with the other sera examined. The serum of a native sheep which had been repeatedly infected over a period of twelve months with several strains of *T. gambiense*, including the strain used in the experiments,

Table II

Showing the action of various sera on a strain of *T. gambiense in vitro* at 37° C.

Strain	Serum	Medium	Concentration of serum in medium	Number of parasites per 256 squares of haemocytometer scale				
				Start	3 hours	6 hours	12 hours	24 hours
Yembuga	Normal human	...	Pure	...	66	60	56	44
		Ringer-glucose solution ...	1/2	...	...	...	...	35
			Control	48	44	42	46	21
Yembuga	Untreated *T. gambiense* sleeping sickness case	...	Pure	...	32	27	...	0
		...	1/2	...	22	28	18	0
		Deactivated serum of sheep 27 and Ringer-glucose solution.	1/5	...	30	28	...	13
		„	1/10	...	...	24	...	10
		„	1/50	...	36	...	...	...
		„	1/100	...	...	35	...	...
		„	Control	45	...	...	...	11
Yembuga	Baboon	...	Pure	...	30	15	12	0
		...	1/2	...	46	...	14	1
		Deactivated serum of sheep 27 and Ringer-glucose solution.	1/5	...	44	48	46	3
		„	1/10	...	...	47	48	5
		„	Control	47	...	...	...	22
Yembuga	Sheep infected many months with several strains of *T. gambiense*	...	Pure	...	0	...	...	...
		...	1/2	...	0	...	...	...
		Deactivated serum of sheep 27 and Ringer-glucose solution.	1/5	...	0	...	...	...
		„	1/10	...	1	0	...	...
		„	1/50	...	35	32	24	3
		„	1/100	...	48	...	...	15
		„	Control	52	...	...	...	16
Yembuga	Fowl	...	Pure	...	40	44	36	30
		Ringer-glucose solution	1/2	...	...	30	32	24
		...	Control	68	62	42	...	32

showed a definite and rapid trypanocidal action. The serum of an untreated native naturally infected in Uganda with *T. gambiense* sleeping sickness and with occasional parasites in his peripheral blood showed very little trypanocidal action, whilst that of a baboon, which was unsuccessfully inoculated on three occasions with all the blood obtainable from monkeys killed while showing a very heavy infection of the strain tested, also had comparatively little trypanocidal action. In determining whether this baboon had become infected or not, large quantities of blood were sub-inoculated on alternate days over a period into monkeys, and, in addition, batches of *G. palpalis* were fed on the animal, but no evidence of the existence of living parasites in the baboon could be found. The observations are of great interest in view of their obvious inconsistency with any thesis that trypanocidal body in the serum is alone responsible for immunity against trypanosomal infection. Fowl's serum was tested on several occasions with both *T. gambiense* and *T. rhodesiense* and was found to support both parasites intact for 24 hours at 37° C. This observation is not so striking as the failure to demonstrate a powerful trypanocidal substance in the serum of baboons, as fowls have been shown on numerous occasions to be susceptible to a mild infection with the pathogenic trypanosomes. The sera of four other sheep, each infected with a single strain of *T. gambiense*, were also tested and were found to have slightly less marked trypanolytic action on this strain than that of the sheep recorded in the Table. These four sheep had not been infected more than a month or so. Whilst the serum of the sheep recorded in the Table was equally efficacious against two other strains of *T. gambiense*, the sera of each of these four sheep appeared to show some specificity of trypanocidal action against its own strain *in vitro*, in that each serum was slightly more rapidly lethal to its appropriate strain. All five infected sheep sera, however, had definitely a lethal action on each of the strains of *T. gambiense* put up against them, whilst the sera of a number of normal sheep did not exhibit any trypanocidal action. In view of the chronic course of the disease in native sheep, it would therefore appear that antibodies are produced after infection, which control the course of the pathological process, and that these anti-bodies are not demonstrable in uninfected sheep in the peripheral blood by *in vitro* tests.

T. RHODESIENSE

The existing laboratory strains of *T. rhodesiense* were studied and a large number of experiments were performed with them. The results of the experiments were frequently somewhat ambiguous, and it was felt that new strains of *T. rhodesiense* fresh from man should be procured as early as feasible on account of the lack of detailed history of the available strains. This was made possible by the kindness of Dr. J. F. Corson, of Tanganyika, who supplied four strains from the Kahama area of Tanganyika. The strains arrived in guinea-pigs and were maintained in these animals, and subsequently in mice, when the latter had been imported and tested on their arrival at the laboratory and at intervals subsequently. Two of the strains were transmitted twice from guinea-pig to guinea-pig by *Glossina palpalis* and their reactions examined after each transmission. Each of the four strains showed definite resistance to the action of pure human serum on its arrival in the first laboratory animal from man.

Table III shows results obtained with the four strains when examined for the action of human serum on them as soon as the animals inoculated from man showed enough parasites in the peripheral blood to enable an experiment to be performed. From this Table it will be seen that a definite proportion of the parasites in each case survives exposure to pure human serum for 24 hours at 37° C. The numbers surviving in the control dilutions of fresh undeactivated rabbit serum in each case are very considerably greater than those in human serum, and in further controls put up with deactivated rabbit serum the numbers were about the same as in the fresh rabbit serum. While the human serum figures show an early marked falling off in numbers by the 6-hour period, after this immediate drop there is a comparatively slight decrease. It would appear that a certain proportion of the parasites is susceptible to the action of human serum and is killed in a short time, whilst the resistant parasites survive the full period of 24 hours at 37° C. Strain C was inoculated into guinea-pigs, monkeys and mice from the guinea-pig which was infected from man, and it was found that inoculation into these various hosts did not appreciably alter the reaction of the parasites to normal human serum.

In each case the proportion surviving at the end of a 24-hour period was about the same as when the original guinea-pig was the source of the material, and again in each case an initial decrease in the number of the parasites was observed. Tested frequently in guinea-pigs up to and including the 5th serial animal, and about 6 months

TABLE III

Showing the effect of normal human serum on four strains of *T. rhodesiense* recovered from the guinea-pigs inoculated directly from man.

Date of experiment	Strain	Concentration of fresh serum in Ringer-glucose solution	Number of parasites per 256 squares of haemocytometer scale					
			Start	3 hours	6 hours	9 hours	12 hours	24 hours
21.10.30	A	Human pure	...	48	34	18	16	15
		1/2	...	...	21	...	14	13
		1/5	72	42	20	...	17	14
...	A	Rabbit pure	...	78	76	...	32	39
		1/2	...	...	...	...	...	28
		1/5	...	...	62	60	...	30
28.10.30	B	Human pure	...	15	8	...	...	7
		1/2	...	26	10	...	...	9
		1/5	82	44	26	23	25	23
...	B	Rabbit pure	...	74	...	...	56	36
		1/2	...	...	...	...	...	20
		1/5	...	...	82	72	...	34
24.10.30	C	Human pure	...	20	5	...	6	5
		1/2	...	...	11	...	9	6
		1/5	100	16	...	14	...	7
...	C	Rabbit pure	...	108	114	...	...	72
		1/2	...	...	118	...	...	86
		1/5	...	122	128	...	...	56
24.10.30	D	Human pure	...	15	12	...	11	9
		1/2	...	...	17	...	15	6
		1/5	90	18	...	20	...	4
...	D	Rabbit pure	...	98	...	...	92	74
		1/2	...	...	112	...	...	76
		1/5	...	120	...	...	...	58

from its isolation from man, this strain was not found to vary greatly in its reaction to normal human serum, the early initial decrease in the number of parasites in the stronger concentrations of human serum, where no other serum was added in the form of medium, remained marked, but no definite evidence of loss of the original degree of resistance could be observed; in the 8th serial guinea-pig, however, very obvious loss of resistance became manifest. Table IV shows the results of an experiment made with material from this animal, and mice inoculated from the animal and used for further experiments gave similar results to those recorded in this Table.

TABLE IV

Showing the effect of a normal human serum on a strain of *T. rhodesiense*, Strain C, recovered from the 8th serial guinea-pig by syringe inoculation, and nine months after isolation from man.

Date	Animal used	Human serum	Concentration of H.S. in Ringer-glucose solution and deactivated serum of sheep 27	Number of parasites per 256 squares of the haemocytometer scale				
				Start	3 hours	6 hours	12 hours	24 hours
26.6.31	Guinea-pig 115A	A.R.D.A.	Pure	...	0	...	...	...
			1/2	...	2	...	...	...
			1/5	...	37	9	0	...
			1/10	...	48	13	0	0
			1/25	...	66	25	2	0
			1/50	...	...	36	7	1
			1/100	...	...	46	11	2
			1/250	...	...	...	26	28
			1/500	...	...	52	32	23 19
			1/1,000	...	...	...	56	38
			Control	58	...	...	...	40

Strain C was studied regularly for a further nine months and was always found to be rapidly susceptible to normal human serum in the stronger concentrations after this period, the action being usually complete in the first three or four tubes by the 3-hour counting period and apparent in further tubes by the 6- or 12-hour counting periods. Strains A, B and D lost their resistance to human serum as did Strain C; Strain A was observed definitely to be susceptible in the 6th, Strain B in the 5th, and Strain D in the 3rd guinea-pigs from man, and during the 5th, 5th and 2nd months respectively after isolation.

In addition to the examinations *in vitro*, some experiments were performed with mice *in vivo*. A dozen mice were inoculated from each of the 5th serial guinea-pigs of Strains A, B, C and D, and were treated with varying amounts of the human sera that had been used in the *in vitro* tests with parasites from these same guinea-pigs. In the case of Strain A, four mice were inoculated with 1·0 c.c., four with 0·5 c.c., and four with 0·25 c.c., of serum intraperitoneally. Although in all these mice the infection had not become heavy at the time of treatment, none was cured, but in all some diminution in the number of parasites in the peripheral blood was noted as a result of the medication ; in one receiving 0·5 c.c. and in one 0·25 c.c., the blood was rendered negative for a period of 48 hours. All the mice died of heavy infections ultimately and within a few days of the controls. In the case of Strain B, two mice, receiving 1·0 c.c. each, were completely cured ; the others, with the exception of two which had 0·5 c.c. each and became negative for three days, were only kept alive for a few days longer than the controls. In the case of Strain C, all the mice became negative, with the exception of two that had 0·25 c.c. each, for three or four days, but relapsed and died in due course ; and again with Strain D all became negative for three or four days, but again relapsed and died. These observations are of interest in showing that none of the four strains was completely resistant to human serum *in vivo* at this stage, as can be seen by the temporary diminution in the number of parasites in the peripheral blood of the treated mice. As the supply of mice was very restricted, it was not possible to continue the observations in greater detail ; but, as the introduction of foreign sera into animals calls into play a number of factors which do not occur *in vitro*, it is doubtful whether much further information of value would have immediately accrued from *in vivo* experiments.

In August, 1931, Dr. Corson again very kindly supplied strains of *T. rhodesiense* from cases in M'wanza, Tanganyika Territory. These strains, six in number, arrived in rats, but were all some months old and had been passed through a number of animals. They were examined soon after their arrival at Entebbe and the results of the examinations are set forth in Table V, together with the histories of the strains as supplied by Dr. Corson. Consideration of this Table shows the fact that none of these strains was then

Showing the action of normal human serum on six strains of *T. rhodesiense* received after they had been kept in laboratory animals for varying periods by syringe passage.

Date examined	Strain	Date isolated from man	Laboratory history	Period out of man	Concentration of normal H.S. in deactivated serum of sheep 27 and Ringer-glucose solution	Number of parasites per 256 squares of the haemocytometer scale				
						Start	3 hours	6 hours	12 hours	24 hours
17.9.31	Bupamba	25.3.30	Goats, guinea-pigs, rats, 1 hyrax and 5 mice.	18 months	Pure	...	9	0	...	...
					1/2	...	13	0	...	...
					1/5	...	32	1	0	...
					1/10	...	30	1	0	...
					1/25	...	33	18	0	0
					1/50	...	38	19	7	0
					1/100	...	...	28	12	4
					1/250	...	...	46	42	18
					1/500	...	...	...	...	26
					1/1,000	...	...	...	...	30
					Control	43	...	...	...	28
14.8.31	Kanawata	24.5.31	4 rats and 2 mice.	3 months	Pure	...	0	...	...	...
					1/2	...	0	...	...	...
					1/5	...	2	0	...	...
					1/10	...	5	2	0	...
					1/25	...	7	4	0	...
					1/50	...	28	3	0	...
					1/100	...	36	17	1	0
					1/250	...	...	23	3	3
					1/500	...	...	24	18	14
					1/1,000	...	...	...	27	17
					Control	38	...	34	...	27
17.9.31	Nangiwa	24.5.31	3 rats and 1 mouse.	4 months	Pure	...	5	0	...	...
					1/2	...	9	0	...	...
					1/5	...	11	5	0	...
					1/10	...	18	3	2	0
					1/25	...	17	12	4	1
					1/50	...	23	14	5	1
					1/100	...	...	24	12	5
					1/250	...	...	28	14	13
					1/500	...	...	27	18	14
					1/1,000	...	...	...	22	23
					Control	32	...	...	...	20
17.9.31	Lambo ...	11.6.31	1 hyrax, 3 rats and 6 mice.	3 months	Pure	...	3	0	...	...
					1/2	...	4	0	...	...
					1/5	...	13	1	...	...
					1/10	...	18	4	...	...
					1/25	...	40	10	...	...
					1/50	...	46	18	3	0
					1/100	...	...	26	16	6
					1/250	...	...	32	22	26
					1/500	...	...	42	38	24
					1/1,000	45	...	...	...	36
21.8.31	Solezi ...	3.5.31	3 rats and 2 mice.	3½ months	Pure	...	1	0	...	...
					1/2	...	0	0	...	...
					1/5	...	30	32	3	0
					1/10	...	46	34	7	1
					1/25	...	...	42	16	2
					1/50	...	...	...	24	10
					1/100	...	...	...	36	28
					1/250	...	...	...	...	32
					1/500	...	...	...	...	34
					1/1,000	...	...	...	...	38
					Control	52	...	...	...	36
14.8.31	Paulo ...	2.7.31	3 rats and 1 mouse.	1½ months	Pure	...	6	0	0	...
					1/2	...	5	3	0	...
					1/5	...	44	21	0	...
					1/10	...	...	52	14	2
					1/25	...	...	...	34	11
					1/50	...	...	...	38	19
					1/100	...	...	...	...	40

human serum fast, but that all were rapidly killed by being exposed to strong concentrations of the serum. The most recently isolated of the strains was 'Paulo,' which had been about six weeks out of man but had been passed through four animals in that time. This strain and 'Solezi,' which was three months old, were killed *in vitro* by concentrations of about 1/5 to 1/10 human serum in 24 hours at 37° C., whilst all the other strains were killed in the same period by concentrations of from 1/50 to 1/100 approximately, Assuming these strains to have been resistant to the action of human serum on isolation, as was the case with the four strains already studied, the resistance has again been seen to diminish materially in a short time and after passage through only a few laboratory animals. Possibly the rats through which they had been passed, and which much more rapidly succumbed to infection, were responsible for the more rapid loss of resistance than was the case in guinea-pigs.

Before the second batch of strains was received from M'wanza, a strain of *T. rhodesiense* used in previous work (Yorke, Adams and Murgatroyd, 1929 and 1930) was obtained from Liverpool. This strain was isolated from man in 1923 and had been maintained since in mice, to which animals it was exceedingly virulent. It was maintained during transit in three guinea-pigs, and was sub-inoculated into mice and into guinea-pigs on arrival. It was usually put up together with other strains as a control during examinations, and was found to be killed by great dilutions of human serum as had already been shown by earlier work. It is of interest here to note that after it had been maintained in 7 guinea-pigs at Entebbe it was inoculated into a sheep which succumbed to a virulent infection in 37 days, during which time parasites were present, frequently in large numbers, after a negative period of 15 days Six hundred and twenty-four *Glossina palpalis* were three times on alternate days fed on this sheep, while the blood was known to be positive by microscopical examination. Of these 624 flies, 409 survived for 22 days from the third infecting feed, to feed twice on each of two clean guinea-pigs. All the flies that died before these final feeds were dissected as they died but no evidence of infection in the gut or glands was found, and the survivors were dissected after their final feeds on the clean guinea-pigs (i.e., on about the

28th to 30th day), with similarly negative results. The guinea-pigs were examined carefully for 6 weeks after the flies had been fed on them but no evidence of infection was found. This experiment would appear to bear out the statement that a strain maintained for many years in laboratory animals by syringe inoculation loses its capacity for infecting fly. Unfortunately, owing to a variety of circumstances, it was not possible to perform the experiment with a larger number of flies, but the number used is sufficient to indicate that at any rate the ' transmissibility ' of the strain is exceedingly slight.

EFFECT OF FLY TRANSMISSION OF A STRAIN OF *T. RHODESIENSE* ON ITS REACTION TO HUMAN SERUM

Three strains were transmitted by fly from guinea-pig to guinea-pig on one or two occasions and their reactions to human serum noted before and after the passages. In Table VI are recorded the results of some experiments of this nature with Strains A, C and D, together with details of the transmissions and the results obtained with parasites obtained from the corresponding guinea-pigs infected by syringe inoculation. It will be observed that, after a single fly transmission, loss of resistance to human serum has taken place, and that this is not the case when the syringe was the means of transference of the parasite. The fact that fly transmission occupied a period of roughly a month must be taken into account, as in the case of Strain D loss of resistance was already obvious in syringe-inoculated animals at the time the fly-infected animal was ready to be used, and in the other strains some indication of loss was to be discerned. This, however, does not alter the fact that in each of these three strains definite loss of resistance to normal human serum *in vitro* was observed after a single fly transmission, and, in the case of Strain A, a second fly transmission apparently caused further loss of resistance, although this loss may have occurred to some extent in the intermediate guinea-pig host. In the 3rd serial guinea-pig by syringe inoculation, however, no such corresponding loss of resistance was observed with this last mentioned strain, neither was there any very marked loss noticed in the 4th or 5th serial guinea-pigs, but in the 8th guinea pig, as shown in Table IV, well-marked susceptibility was apparent,

TABLE VI

Showing the effect of fly transmission on the reaction to human serum of some strains of *T. rhodesiense*.

Date of experiment	Strain	Animal and how infected	Concentration of H.S. in deactivated serum of sheep 27 and Ringer-glucose solution	Number of parasites per 256 squares of the haemocytometer scale				
				Start	3 hours	6 hours	12 hours	24 hours
19.11.30	A	Guinea-pig 18B; by syringe from 1st guinea-pig from man.	Pure	...	42	46	44	30
			1/2	...	48	54	46	33
			1/5	...	84	64	56	34
			1/10	...	...	...	48	50
			Control	82	...	78	...	52
24.12.30	A	Guinea-pig 22A; by fly from 1st guinea-pig from man.	Pure	...	0	...	...	...
			1/2	...	0	...	...	...
			1/5	...	56	14	...	0
			1/10	...	72	34	...	0
			1/25	...	...	54	...	14
			1/50	...	...	56	...	27
			1/100	...	...	53	...	22
			1/500	...	...	...	...	26
			Control	68	...	...	...	40
3.3.31	A	Guinea-pig 22B; by fly from above guinea-pig (22A), 2nd fly passage.	Pure	...	12	0	...	...
			1/2	...	14	0	...	...
			1/5	...	78	18	1	...
			1/10	...	86	46	0	...
			1/25	...	...	68	10	0
			1/50	...	...	84	12	16
			1/100	...	...	...	36	24
			1/500	...	...	...	120	32
			Control	130	...	...	...	64
19.11.30	C	Guinea-pig 24B; by syringe from 1st guinea-pig from man.	Pure	...	38	44	43	36
			1/2	...	60	56	64	52
			1/5	...	...	60	...	54
			1/10	...	...	...	...	48
			Control	62	...	...	...	56
9.1.31	C	Guinea-pig 24B; by fly from 1st guinea-pig from man.	Pure	...	1	0	...	...
			1/2	...	5	1	...	...
			1/5	...	48	18	2	0
			1/10	...	58	20	8	5
			1/50	...	72	28	19	15
			1/100	...	...	42	26	18
			Control	68	...	...	...	36
	D	Guinea-pig 21B; by syringe from 1st guinea-pig from man.	Pure	...	10	0	...	...
			1/2	...	5	0	...	...
			1/5	...	8	5	1	0
			1/10	...	33	18	6	2
			1/50	...	...	74	34	14
			1/100	...	...	...	32	30
			Control	66	...	...	...	46
	D	Guinea-pig 25B; by fly from 1st guinea-pig from man.	Pure	...	0	...	...	...
			1/2	...	2	...	...	...
			1/5	...	32	18	0	...
			1/10	...	36	19	10	1
			1/50	...	78	52	16	7
			1/100	...	...	56	20	15
			Control	72	...	...	...	34

T. BRUCEI

Two strains of *T. brucei* were studied *in vitro*. The first, referred to as the N'songezi strain, was obtained by me in the Ankole district of Uganda in May, 1931. No human cases of trypanosomiasis are known to occur in this district, but all the domestic stock has been evacuated from the area on account of nagana, and the country consists of bush populated by large herds of many species of game, and is heavily infested with *Glossina morsitans*. Three dogs were taken into the area and on two of them between 1,000 and 2,000 wild *morsitans* were fed as they were caught over a period of ten days. The third animal was left in the district and allowed to wander at random with boys engaged in clearing bush. All three dogs developed infections, those on which caught flies were fed showed parasites inside two weeks and the third animal died within 3 months of its entry into the area. The strain examined was obtained from one of the first two animals referred to, and was subsequently kept in guinea-pigs and mice. It resembled *T. brucei* in its morphology and in its virulence to laboratory animals. The second strain of *T. brucei* was supplied by Dr. Corson and had been originally obtained from Mr. Hornby in Tanganyika. It is referred to as Hornby's virulent *brucei* and was recovered from a heifer in April, 1927, and had since been maintained in rats, guinea-pigs, rabbits and some goats. In Table VII are set forth some typical results of experiments to determine the reactions to normal human serum of these two strains. From this Table it can be seen that both strains are definitely susceptible to the action of normal human serum *in vitro*, but that their susceptibility is no more marked than that of strains of *T. rhodesiense* after a few months' passage in laboratory animals; it is consequently difficult to understand why such strains should not be capable of infecting human beings with lowered resistance, as judged by the trypanocidal titres of their sera, if immunity to infection can be estimated by such means. That the second of these strains, however, is not infective to man is indicated by Corson's experiment (1932), in which flies infected with it were allowed to feed on two European volunteers, neither of whom developed an infection.

TABLE VII

Showing the reaction to normal human serum of two strains of *T. brucei.*

Date of experiment	Strain	When isolated	Concentration of human serum in deactivated serum of sheep 27 and Ringer-glucose solution	Number of parasites per 256 squares of the haemocytometer scale				
				Start	3 hours	6 hours	12 hours	24 hours
12.5.31	N'songezi *brucei*	April, 1931	Pure	...	42	0	...	...
			1/2	...	48	0	...	...
			1/5	...	59	30	1	0
			1/10	...	62	42	9	0
			1/25	...	...	52	15	5
			1/50	...	...	48	21	11
			1/100	...	...	50	46	25
			1/250	...	...	...	...	39
			1/500	...	...	...	...	40
			1/1,000	...	...	...	...	46
			Control	54	...	...	...	48
18.8.31	Hornby's virulent *brucei*	April, 1927	Pure	...	5	0	...	...
			1/2	...	14	0	...	...
			1/5	...	44	28	0	...
			1/10	...	46	32	1	0
			1/25	...	44	46	0	0
			1/50	...	...	48	3	0
			1/100	...	...	52	19	9
			1/250	...	...	...	39	11
			1/500	...	...	70	...	32
			1/1,000	...	...	...	38	36
			Control	68	...	74	52	46

DISCUSSION

Whilst the complete resistance to the action of pure human serum is what would be expected with *T. gambiense*, the obvious inferences to be drawn from the experiments with *T. rhodesiense* strains are that human serum fastness occurs in a strain of parasites which has gained a footing in man, due to initial lowered resistance on the part of the individual, and that once so established it may become potentially capable of infecting normal healthy individuals by mechanical means, but not by means of cyclical development in *Glossina*. This hypothesis would supply a satisfactory explanation of the epidemiology of endemic and epidemic sleeping sickness due to *T. rhodesiense*. Corson (1932), however, since the above work was done, has published a record of his striking experiments performed with two of the strains investigated by me in the course of this work. He successfully infected himself with the strain 'Bupamba' by sub-cutaneous inoculation, after the strain had been maintained in goats, sheep, rats and guinea-pigs by syringe passage for 19 months, and had then been transmitted cyclically by fly. He records of this strain that: 'After maintenance in goats for three and six months it showed a greater susceptibility to human serum, as tested in subinoculated rats, than fresh strains of *T. rhodesiense* from man. The susceptibility to human serum was again tested sixteen months after its isolation from man, and was found to be much the same as at six months.' I myself found it to be definitely killed by human serum to a dilution of about 1/25 in 6 hours and to about 1/100 in 24 hours *in vitro* at 37° C. after 18 months in experimental animals (cf. Table V). It would, therefore, appear from this single experiment of Corson's that human serum fastness is not necessarily a criterion of capacity to infect man, assuming that Dr. Corson possesses the normal amount of trypanocidal body in his serum. In the case of the baboon, the converse has already been shown to be the case, namely, that complete immunity to infection is not coincident with high trypanocidal titre of the serum; and, again, it is above shown that in the case of *T. brucei* the resistance to the action of normal human serum is not as great as would be expected from the observations on an old laboratory strain. The further pursuit of such lines of investigation should materially assist in clarifying the position as to the power of mutation of one form of these parasites into another, and also in elucidating a number of, so far, unsatisfactorily explained points in the epidemiology of the conditions caused by them. The end point in all experiments of this nature must be man himself, as, without being in a position to put hypotheses to the crucial test on a reasonable scale, it will be difficult even to approach finality on the subject.

SUMMARY

1. A technique, already described in previous publications, was employed to determine the action of normal human serum on strains of *T. gambiense*, *T. rhodesiense* and *T. brucei in vitro*. Certain modifications were introduced, so that guinea-pigs could be used instead of mice, as in the original work, until the latter animals became available.

2. The deactivated sera of rabbits, sheep, goats, guinea-pigs and monkeys (*Cercopithecus* sp.) were all found to provide suitable media for the maintenance of the pathogenic trypanosomes for periods up to 24 hours *in vitro* at 37° C.

3. No differences in the amount of trypanocidal body present in the sera of healthy natives and those of healthy Europeans could be detected. Filarial infection did not affect the amount present in native sera.

4. Four strains of *T. gambiense* were studied over a period of 12 months, and no evidence of susceptibility to the action of human serum could be detected in any of them throughout this period. Passage of the strains through fly did not affect their complete resistance.

5. A strain of *T. gambiense* was tested against the serum of a case of sleeping sickness, the serum of a multiple-infected sheep, and the sera of a baboon and some fowls. All were trypanocidal to a greater or less degree, with the exception of the fowl serum, that of the sheep having the most pronounced lytic action. The sera of four other more recently infected sheep were also trypanolytic, and some degree of specificity of action on their appropriate strains was discerned.

6. Four strains of *T. rhodesiense* in the guinea-pigs inoculated from man showed a definite degree of human serum-fastness. Their resistance was not absolute, but a proportion of the parasites was rapidly susceptible to the higher concentrations of normal human serum, while the remainder was resistant.

7. Transference of strains of *T. rhodesiense* through small laboratory animals by means of syringe inoculation rapidly caused loss of human serum resistance, and by the 3rd, 5th, 6th and 8th

serial animals evidence of complete susceptibility to dilutions of serum down to at least 1/10 was apparent in 12 hours at 37° C.

8. After a single cyclical transmission of three strains of *T. rhodesiense* through *Glossina palpalis*, considerable loss of resistance to the action of human serum was observed. A second cyclical passage through fly caused some further loss of resistance.

9. Six further strains of *T. rhodesiense*, first examined after each had been passed through several animals over a period of a few months, were studied and found susceptible to human serum to a definite degree.

10. Two strains of *T. brucei*, one freshly isolated from an area devoid of human trypanosomiasis, and the other a four-year-old strain, were susceptible to the action of human serum, but to a less degree than would be anticipated from a study of old strains of *T. rhodesiense*.

11. From an examination of the data presented here, and in view of more recent experiments by Corson (1932), it is clear that human serum sensitiveness is not an indication of lack of ability to infect man, and that, conversely, immunity depends on further factors with which we are not fully conversant.

12. An observation is recorded that an old laboratory strain of *T. rhodesiense*, ten years in small animals and not in contact with fly during that time, was rapidly fatal to a native sheep when inoculated sub-cutaneously, and failed to infect at least 400 *Glossina palpalis* when fed on this animal while its peripheral blood was shown to be positive microscopically.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS *MUSCA*, BASED ON A COMPARATIVE STUDY OF THE MALE TERMINALIA

II. A PRACTICAL GUIDE TO THE PALAEARCTIC SPECIES

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The comparative study of the male terminalia of 41 species of *Musca* as described in the first paper (1932) of this series has not only demonstrated the great value of these structures in discovering the true affinities and relationships of the species, but has also afforded the only certain means of understanding the apparently anomolous and atypical forms. This study has also greatly facilitated the present revision by clearing up the identity of closely related species which was not otherwise possible.

In the present paper is given a practical guide to the 16 species of the Palaearctic region known to me at present. In order to facilitate the ready recognition of the species, not only from this region, but also from the other zoo-geographical areas, which will be dealt with in succeeding papers, it is necessary at the outset to explain certain diagnostic characters which will frequently be referred to in the keys and descriptions. The technique employed in making permanent microscopic mounts of the male terminalia is also described. These explanatory notes will not only be useful in this genus but also in others to be dealt with later.

NOTES ON CHARACTERS OF USE IN IDENTIFICATION

HEAD. VERTEX. By the term vertex is here understood the space between the inner margins of the eyes on the anterior part of the head in both sexes. It is commonly referred to as the front or frons. In the male it is always narrower than in the female. It extends from the upper margin of the head to the bases of the antennae in the middle, and down the sides to about the level of the end of the third antennal segment. In flies which have been pinned before the head has properly hardened, and the ptilinal suture closed after the withdrawal of the ptilinum, the vertex is often collapsed, and then a very erroneous idea of its width is gained.

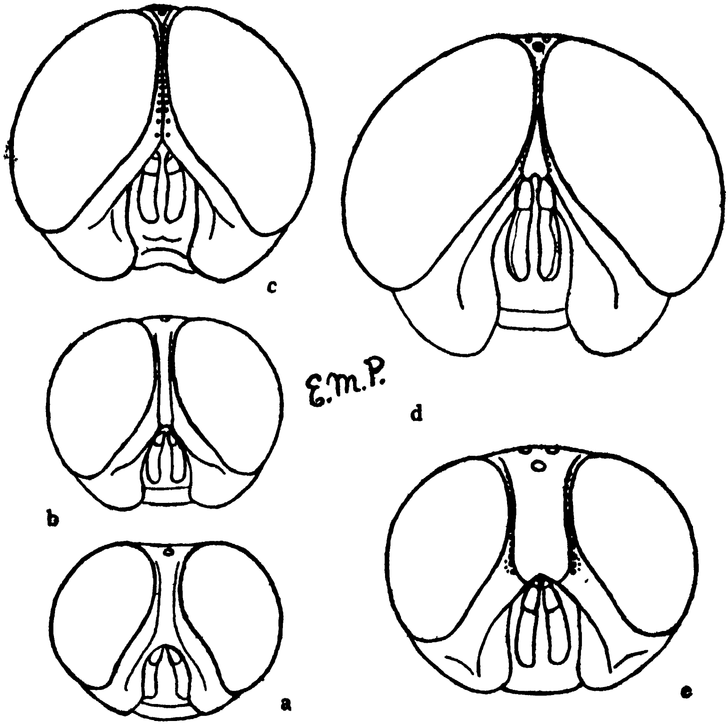

FIG. 1. *a.*—Head of male *M. sorbens* (Sierra Leone), showing narrow type of vertex; *b.*—Head of same (India), showing slightly narrower vertex; *c.*—Head of male *M. ventrosa*, showing linear vertex; *d.*—Head of male *M. bezzii*, also showing linear vertex; *e.*—Head of male *M. domestica*, showing the wide type of vertex.

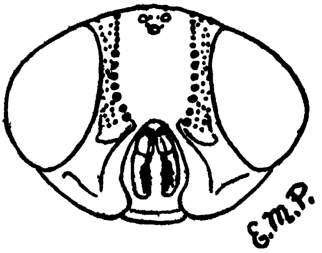

FIG. 2. Head of female *M. domestica*;—note width of vertex and double row of outer vertical bristles.

MALE. There are three main types of vertex in the males which will be referred to as follows:—(1) Vertex wide, eyes widely separated. EXAMPLES. Vertex of the male *domestica* (fig. 1, *e*), *vicina*, *inferior* and *fletcheri*. In *domestica* (fig. 1, *e*) the vertex is approximately half the width of an eye, while in *vicina* it is about one third. The vertical stripe, a broad dark stripe, which extends almost the whole length of the vertex, is well developed in all the above four species, and is bordered by the usual convergent, inner vertical bristles. (2) Vertex narrower, eyes well separated. EXAMPLES. The vertex of the male of *sorbens* (fig. 1, *a*, *b*), *nebulo*, *lucidula*, *vitripennis*, *crassirostris*, etc. In these species the vertex is narrower, varying from one-fourth to one-seventh the width of an eye. (3) Vertex linear, eyes closely approximated. EXAMPLES. The vertex of the male of *bezzii* (fig. 1, *d*), *bakeri*, *autumnalis*, *larvipara*, *hervei*, *ventrosa* (fig. 1, *c*) and others. In these species the eyes are separated by a narrow to a very narrow vertical line; this is the type of vertex which is common to most of the large species of the *lusoria* group.

FEMALE. In the females the vertex is always wide, and the sides are usually of a creamy or yellowish-white colour. There is in most a broad, dark vertical stripe which varies in width in the species. It is very wide, and has bulging sides in *domestica* (fig. 2), *bezzii* (fig. 3, *a*) and others, whereas in *lucidula* and *albina* (fig. 3, *b*) it is narrow and has straight sides.

VERTICAL BRISTLES. In both sexes the vertical stripe is bordered on both sides by a row of 7 to 12 convergent, inner vertical bristles which vary in size and even in position in the species. On each side of the vertical stripe from about the level of the bases of the antennae to about the level of the ocellar triangle, there are in the female one or more rows of smaller bristles and hairs, the outer vertical bristles and hairs. In *tempestiva*, *vitripennis*, *conducens* (fig. 4), they are arranged in a single row; in most of the species of the *lusoria* group they are arranged in two or three rows (fig. 3, *a*); in *albina* (fig. 3, *b*) and *lucidula*, especially the former, there are four or even five rows of small hairs arranged somewhat irregularly. The width of the vertex and that of the vertical stripe, and the arrangement of the outer vertical bristles and hairs, are useful subsidiary characters in identification.

Eyes. Vestiture. In the majority of species there are no hairs between the facets, and the eyes are said to be bare. In some, however, the hairs are numerous and long, and are easily detected, as, for instance, in the male of *vitripennis*, of *interrupta* and *albina*, and in both sexes of *dasyops*. In both sexes of *gibsoni* and *hervei*, on the other hand, the hairs are scanty and very small, and can only be detected with a high magnification. Facets. In many species, as for instance *xanthomelas*, *spinohumera*, *sorbens* and others, there is an area of large facets on each side of the vertex about the middle.

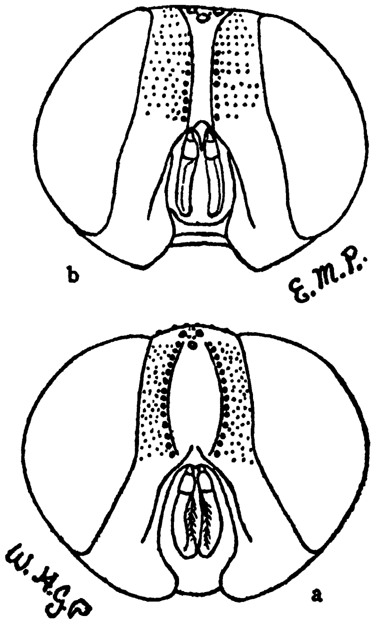

Fig. 3. *a.*—Head of female *M. bezzii*, showing three rows of outer vertical bristles; *b.*—Head of female *M. albina*, showing the narrow vertical stripe and 4-5 rows of outer vertical hairs.

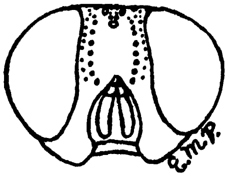

Fig. 4. Head of female *M. conducens*, showing the single row of outer vertical hairs.

Palps. The palps are long and vary to some extent in the species; they are always more slender in the male. In the majority they are black, but in *crassirostris*, *inferior* and *senior-whitei*, and to some extent in *conducens*, they are yellowish.

PROBOSCIS. The proboscis is retractile in all the species. It exhibits the early phases in the evolution of the non-retractile, blood-drawing proboscis of *Stomoxys* and *Glossina*. A short comparative account with illustrations of the proboscis of the seven species which exhibit these changes has been given in another paper (1933). The bulbous mentum of *crassirostris* (fig. 5), and to a less extent that of *inferior*, is a most useful subsidiary character in the identification of these two species. It is quite common to find specimens of both with the proboscis extended, the labella drawn back and the prestomal teeth everted. In some species the labella are longer and slightly narrower as in *tempestatum*.

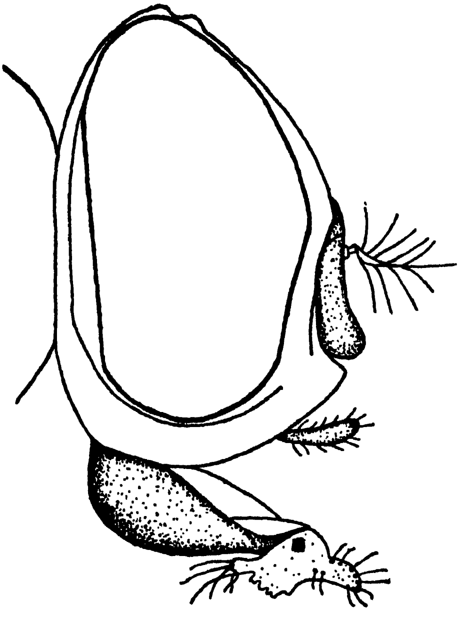

FIG. 5. Head of female *M. crassirostris*, in side view, showing the bulbous mentum.

THORACIC MARKINGS. MESONOTUM. In the majority of the species as exemplified by *domestica*, *nebulo*, *crassirostris*, *lusoria*, *bezzii*, etc., the mesonotum is marked with four dark stripes, two on each side of the middle line; these species are referred to in the keys as 'mesonotum with four dark stripes.' In a few, as for instance *sorbens*, *vetustissima*, *tempestatum*, *fasciata*, etc., the stripes of each side are fused to form a single one, and these species are referred to as 'mesonotum with two dark stripes.' In the female *sorbens*, the single stripe of each side is divided Y-like in front of the suture, but behind it there is only one stripe as in the male. In some species the inner pair are distinctly and constantly narrower than the outer pair. In the males of a few species such as *albina*, *vitripennis*, *lucidula*, and to some extent in *tempestiva*, the mesonotum is metallic, and there are no visible markings, except faint ones as in *tempestiva*. These mesonotal markings are most useful in eliminating the species in the keys. When looking for them it is most important to examine the mesonotum in a good light and from all angles, and care should be taken not to confuse the light grey stripes between the dark ones, as is so frequently done by the beginner.

THORACIC BRISTLES. The number and arrangement of the mesonotal bristles are of some use in identification, provided that it is remembered that, as in the case of bristles elsewhere, they are variable in size, number and arrangement. There are normally on each side three dorsocentrals anterior to the mesonotal suture, the presutural, and four posterior to it, the postsutural. There is only one pair of acrostichals at the posterior end of the mesonotum. These bristles may be reduced in size and number, and may even be wanting in species in which they are normally present. In *crassirostris* there are only two presutural dorsocentrals and two behind the suture, one as a rule being small. In *albina* there is a further reduction, there being only one presutural dorsocentral and one postsutural, and the acrostichals are wanting. The sternopleurals, which are always arranged 1 : 2 (one anterior and two posterior), are present in all the species with the single exception of *albina*, in which they are wanting in both sexes. It is, of course, necessary to know where to look for these thoracic bristles, and the reader is referred to the drawings illustrating the chaetotaxy of the thorax in Part I of my book, 'Insects, Ticks, Mites, etc.'

WING. VENATION. In all the species except *lucidula*, vein M_{1+2} joins R_{4+5}, and cell R_5 is closed. Although in the majority of the species the bend of vein M_{1+2} is an elbow bend, in *tempestiva* and *tempestatum* the bend is more rounded.

WING PILOTAXY. The presence or absence of small bristles and hairs on the ventral surface of vein R_{4+5} is a subsidiary character. These hairs may be limited to the part of the vein proximal to the radio-medial cross vein, or they may extend up to the level of the bend of M_{1+2}. In most of the species of the *domestica* and *sorbens* groups they are localised to the proximal part of the vein, with the single exception of *planiceps* in which they are present all along the vein up to the bend of M_{1+2}. In most of the species of the *lusoria* group, on the other hand, the bristles are present all along the ventral surface of R_{4+5} to the bend of M_{1+2}. It should be remembered that these small bristles are readily detached when the flies are handled, the calyces then alone indicating their presence. It is also not uncommon to come across specimens of species in which these bristles (which they normally possess) are wanting. When looking for the bristles it is necessary to use a strong lens and to examine the ventral surface of R_{4+5}. The hairs on the upper surface of R at its base are very variable and are commonly dislodged. SQUAMA. In one species, *inferior*, there is a number of long, delicate hairs on the upper surface of the squama about the middle ; this is a useful character in separating *inferior* from *fletcheri*, which resembles it somewhat and in which they are wanting.

ABDOMEN. DORSAL SURFACE. As already pointed out in the first paper (1932), only four terga are visible from the dorsal surface, the apparent first being a compound sclerite consisting of the fused first and second terga. In the keys and descriptions which follow, this apparent first tergum is referred to as terga 1 and 2. The fifth tergum (apparent fourth) is the last visible one, and will be referred to as tergum 5. The sixth and remaining terga are tucked away on the ventral surface of the abdomen in the male, and in the female form the ovipositor. The visible terga may be of uniform colour as in *ventrosa*, or, as in the majority of the species, they are marked with stripes, bands, silvery spots, etc. It should be particularly noted that the descriptions of these colour markings are drawn up with the head of the specimen directed towards the observer, and, therefore, when using these descriptions the specimen must be viewed that way. VENTRAL SURFACE. Normally the five visible sterna are orange in colour, but in quite a number of males the first is dark brown, as is also the distal end of the fifth, especially at the sides. In the male of *larvipara* and *autumnalis*, and in those of several others, the second sternum is also dark brown to black. In the male of *hervei* (fig. 6) and that of *bakeri*, all the sterna are black, and in the former the adjoining edges of the terga are also black. In the females the sterna are either orange, dark grey or black.

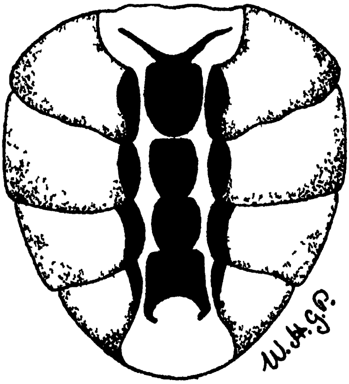

FIG. 6. Ventral view of abdomen of male *M. hervei*, to show the black sterna and adjacent edges of terga.

MALE TERMINALIA. THE MAKING OF PERMANENT MOUNTS. As the terminalia are of the first importance, not only in discovering the affinities and relationships of the species, but also in separating closely allied forms, it is necessary to describe briefly the technique which has now been successfully employed in making hundreds of permanent mounts of the parts. If a number of specimens are available, either the entire fly or only the abdomen is placed in a small dish in 1 in 10 per cent. caustic potash. In removing the abdomen, care is necessary in order to detach the first sternum without tearing it. If, on the other hand, only one specimen is available, one of two methods can be employed in removing the terminalia so as to avoid mutilating the specimen more than is necessary. (1) With a pair of fine, sharp scissors a slanting cut is made across the end of tergum 4 (apparent third), making sure to include the fifth sternum. (2) With a fine needle, a good light and a binocular microscope, the fifth tergum (apparent fourth) can be slowly separated from tergum 4 (apparent third), so as to include the fifth sternum as well. I have found it most unsatisfactory to try and scoop out the terminalia with a blunt bent needle, as almost all the parts get damaged beyond repair. If, when making the cut or separating the fifth tergum with the needle, the abdomen should come off, as it often does in dry specimens, it should be allowed to fall into a small dry dish and the dissection completed; the abdomen can then be replaced with the aid of a minute drop of Canada balsam.

The specimen, whatever it may be, is left in the caustic potash until all the soft parts are destroyed and the chitin sufficiently decolourized, the amount of the latter depending on the specimen. The potash is now removed with the aid of a pipette and the specimen thoroughly washed in several changes of water. It is now passed through two changes of 70 per cent. alcohol to absolute alcohol, in which it is left for several hours. The absolute alcohol is now replaced by clove oil, and when the specimen is cleared the parts are dissected off with the aid of fine needles, a good light and a binocular microscope. It will be noted that the specimen is not removed from the glass dish during this preparatory treatment, the various fluids being changed with the aid of pipettes.

If the entire fly has been so prepared, the abdomen is first dissected off. The first five sterna are now detached from the terga. The fifth tergum bearing the terminalia is next separated from the fourth. The fifth, sixth and seventh terga are next dissected away. The anal cerci are then separated from their attachments to the tenth tergum, and in doing so great care must be taken not to damage or detach the small distal segment of the ninth coxite, which is very small in *Musca* and allied genera. The long lateral processes of the tenth tergum are detached from their articulations with the ninth tergo-sternum, as are also the proximal segments of the ninth coxite. The distal segment of the ninth coxite is now separated from the cercus of its side and left attached to the large, hand-like, proximal segment. The phallosome and parameres are now left attached to the ninth tergo-sternum, and the most difficult part of the dissection is their removal intact. This is best accomplished by turning the ninth tergo-sternum over and passing a fine needle under the apodeme of the phallsome, at the same time steadying the ninth tergo-sternum with the other needle; the phallsome and attached parameres can then be gently detached together so that the latter retain their natural relationships to the former. When, as in the case of the single specimen, the fifth tergum and sternum have been removed and prepared as noted above, the fifth sternum is detached from the seventh tergum, and the remainder of the dissection continued as described above.

When all the parts of the terminalia have been dissected off, a small drop of Canada balsam is placed on a glass slide, and each part is lifted out on a needle and placed in it, and oriented in a suitable manner. The slide is kept covered in a safe place until the balsam has set, and, when the parts have become fixed in the desired position, some fresh balsam is added, and the preparation is left for several days. It often happens that the parts have moved out of the desired position, and in that case they must be re-arranged again; a drop of xylol often helps in softening the balsam sufficiently to allow of their being moved with the aid of fine needles. It often takes days to get a part oriented in the position in which it is desired to illustrate it. Finally, when all the parts have set in the required positions, fresh fluid balsam is placed over each and a coverslip is gently lowered on to them. In this way excellent permanent mounts of all the parts are obtained without any compression, and they can be studied with high powers of the microscope. I have in this way made permanent mounts of the male terminalia of all but those of three species of *Musca*. In the illustrations of the various parts of the terminalia accompanying these papers, all the phallosomes and parameres have been drawn to the same scale, but not the parts of these illustrated around them ; similarly all the anal cerci and all the fifth sterna have been drawn to the same scale. The characters of taxonomic value are :—The size and structure of the anal cerci, especially that of the outer and inner ends of their free borders, the size and structure of the fifth sternum and especially that of its processes, the structure of the phallosome and its posterior process, the structure of the paramere, and especially its posterior part, and lastly the structure of the two segments of the ninth coxite.

THE USE OF THE MALE TERMINALIC CHARACTERS IN FRESH SPECIMENS. In only a very few species of *Musca* can a certain identification be made in the case of fresh specimens without any dissection by drawing back the anal cerci with a needle, and noting the structure of their free margins, In *lusoria* (Ethiopian) and *spinohumera* (Oriental), the anal cerci have large, stout, projecting nipple-like processes at both ends of the free border (see p. I, fig.

15, *b*) which can readily be detected by this simple procedure. The male *larvipara* can also be distinguished from that of *autumnalis* by noting the structure of the posterior processes of the fifth sternum and the free margins of the anal cerci. As the other parts of the terminalia, the phallosome, its posterior process and the parameres are extremely small, it is quite impossible to make out the details of their structure by examining them in this way. That this is so it is only necessary to refer to three illustrations, Nos. 546, 548 and 559 in Schnabl and Dziedzicki's work (1911), in which are depicted the anal cerci, ninth coxite, ninth tergo-sternum and phallosome (without parameres) of *domestica*, *autumnalis* and *larvipara*. These illustrations of the phallosome of these three species are caricatures of the originals. It just happens that in these three species it is possible to identify them on the characters of the anal cerci and the posterior processes of the fifth sternum without examining the phallosome and parameres. It would, however, be quite impossible to distinguish species in which the anal cerci and fifth sternum are very similar by examining the phallosome (its posterior process and the parameres) while still attached to the abdomen. The minute differences in these parts, on which the determination depends in the case of closely allied species, can only be detected with certainty when they are dissected off and mounted as described above. It would be impossible, for instance, to distinguish *domestica* from either *vicina* or *nebulo*, or *crassirostris* from *lucidula*. Mounting the parts of the terminalia by flattening them out under a coverslip after dissecting them off is equally unsatisfactory, and may lead to erroneous conclusions, as I know from past experience.

KEYS TO THE SPECIES

ADULTS. The following keys to the sexes of the species of the Palaearctic region are based on external characters which can be noted with the aid of a lens magnifying ten diameters. They have been drawn up after examining long series of most of the species, and variations have, therefore, been noted. MALES. In the case of the key to the males no difficulty should be experienced in recognising most of them, for there are many reliable characters of use in doing so. The male *autumnalis* is, however, extremely difficult to distinguish from the male *larvipara* on characters other than the terminalia. It is no wonder then that they have long been confused, and, although Portchinsky (1885) suspected that his viviparous variety of *autumnalis* (*corvina*) was distinct from what he considered was the oviparous form, it was not until Schnabl and Dziedzicki (1911) pointed out the differences in the anal cerci and the posterior processes of the fifth sternum that their true identities were established. I have a series of males identified as *autumnalis* (*corvina*) by the late Professor Bezzi and the late Dr. Lichtwald, which on examination of the terminalia prove to be *larvipara*. FEMALES. It is extremely difficult to draw up a satisfactory key to the females, as there are very few characters available for separating them, and many of them, even in this limited fauna, are very alike and difficult to distinguish. There should, however, be no difficulty in recognising the females of such species as *albina*, *lucidula*, *tempestiva*, *vitripennis*, *sorbens*, *ventrosa* and *crassirostris*. It is, however, surprising to find how often *crassirostris* is wrongly identified. Personally I have always found it one of the easiest species to recognise, the bulbous mentum (fig. 5) affording a striking character, apart from its olive grey colour. It is almost impossible to distinguish the female *domestica* from the female *vicina*, but as a rule the latter is smaller and the abdomen more orange. The females of *convexifrons*, *hervei*, *mesopotamiensis*, *autumnalis* and *larvipara* are all so similar that it is equally difficult to separate them. The first two are Chinese species, though *hervei* is also found in North India. The eyes of *hervei* are minutely haired, as in the male, whereas they are bare in *convexifrons*. When in China I collected a single male of *autumnalis* at Tsinan, Shantung, so that Chinese entomologists will have to distinguish it from these two species which closely simulate it. In *convexifrons* there is usually a row of bristles on the ventral side of vein R_{4+5}; these are never present in *autumnalis*. *M. convexifrons* is larviparous, whereas both *hervei* and *autumnalis* are oviparous, and the latter has a white puparium. *M. mesopotamiensis* is only found in Iraq, Arabia and South Persia, as far as I know at present; the female has a narrow vertical stripe, and the outer vertical hairs are arranged in a single row. It is impossible on external characters to distinguish the female *autumnalis* from that of *larvipara*; the former is oviparous and has a white puparium, whereas the latter is larviparous, and has a dark brown puparium.

LARVAE. At present the larvae of only nine species are known to me, so that it is not worth while giving a key for their identification. The important characters of use in distinguishing the larvae in this genus are as follows:—The shape and general character of the posterior spiracular plates, the width of the space separating them, the character of the peritreme, and the structure of the spiracular slits. In all the species the plates are more or less D-shaped, the straight edge of the D being inwards, and the three slits are sinuous. It is interesting to note that the plates in the higher groups, and especially in the lusoria group, are large and more densely chitinised so that no definite peritreme can be made out, and the slits are often very narrow, long and tortuous. The number of finger-like processes of the anterior spiracles are not very reliable characters as they are very variable even on the two sides of the same larva. All the posterior spiracles illustrated have been drawn to the same scale, and are, therefore, comparable.

KEY TO THE MALES OF THE PALAEARCTIC SPECIES BASED ON EXTERNAL CHARACTERS

1. Species with metallic mesonotum and without stripes 2

Species with non-metallic mesonotum and with dark stripes ... 5

2. Eyes hairy 3

Eyes bare 4

3. Eyes with numerous long hairs. Sternopleurals present and dorsocentrals and acrostichals not much reduced. Abdomen light orange. Terga 1 and 2 dark green, appearing black, in middle and along anterior border, sides light orange; tergum 3 orange with a broad, median, dark green stripe, expanding T-shaped anteriorly; tergum 4 greyish white in middle and at most of sides, lateral margins yellow, either a small dark spot on middle of posterior border, or spot much larger and almost meeting posterior end of dark stripe on tergum 3; tergum 5 often with an indistinct dark spot about middle, or only dark on posterior border .... *vitripennis*

Eyes with hairs less long and numerous. Sternopleurals wanting, and mesonotal bristles much reduced. Abdomen orange; tergum 1 and 2 either entirely orange, or with a faint median dark spot, or a band which may spread on to the next tergum; tergum 3 with a small (often conspicuous) dark spot, which may spread forwards and form an incomplete, median stripe; tergum 4 with a large median, greyish yellow patch not as large as in *vitripennis*, and a small dark posterior spot; tergum 5 with a large, median, greyish yellow patch, and a pair of small admedian black spots......... *albina*

4. A small species. Mesonotum bright metallic green, prescutum with some white dusting, and sometimes faintly marked stripes. Abdomen mainly light orange ; terga 1 and 2 light orange, either with a metallic patch only on anterior border on each side of middle line, or in addition a metallic spot at middle of posterior border tending to join anterior patch ; tergum 3 either entirely orange or with an incomplete, dark median stripe ; tergum 4 light orange with a metallic green patch on middle of posterior border, and sometimes a faint indication of a median dark stripe as well ; tergum 5 metallic green .... *lucidula*

A small species. Mesonotum with dark metallic sheen. Abdomen greyish green with bands and stripes; terga 1 and 2 dark brown to black........................ *tempestiva*

5. Larger dark species. Sterna 1-5 black, also adjacent edges of terga 1-4. Eyes scantily and minutely haired. Mesonotum grey with four well-marked dark stripes. Abdomen mainly dark orange with dark bands and stripes. China, N. India... *hervei*

Sternites not all black and adjacent edges of terga not marked as above. Eyes bare........................ 6

6. Medium-sized grey species. Mesonotum with *two* broad, dark stripes. Terga 1 and 2 orange, sometimes dark orange with an almost complete, dark median stripe, or terga 1 and 2 entirely dark brown to black........................ *sorbens*

Mesonotum with *four* dark stripes 7

7. Abdomen orange without markings........................ *ventrosa*

Abdomen either orange, partly orange, or not at all orange, but always marked with stripes, bands and silvery or creamy spots 8

8. Eyes widely separated. Vertex one-third to one-half width of an eye 9

Eyes either well separated or closely approximated 10

9. Vertex approximately half width of an eye. Mesonotum dark grey with four well-marked dark stripes of about equal length. Abdomen dark orange proximally and distally yellowish grey ; tergum 1 and 2 orange with a broad, median black stripe spreading out anteriorly ; tergum 3 dark orange with a broad, black median stripe bordered by a silvery stripe on each side ; tergum 4 greyish yellow with a broad, black median stripe ; tergum 5 yellowish with some dark areas in middle .... *domestica*

Vertex approximately one-third width of an eye. Abdomen much lighter orange .... *vicina*

10. Eyes well separated 11

Eyes closely approximated 13

11. Medium-sized, sometimes rather small, greyish or olive green species. Palps yellow. Mentum deep, strongly chitinised, forming a characteristic bulb. Mesonotum greyish yellow with four, rather narrow, dark stripes. Abdomen greyish green .... *crassirostris*

Medium-sized to larger species. Palps black. Mentum not bulbous 12

12. A medium-sized species. Mesonotum grey with four dark stripes. Abdomen light orange; terga 1 and 2 with a broad, median dark stripe expanding anteriorly to form a dark band across anterior half; tergum 3 with a broad, median dark stripe also expanded a little anteriorly, bordered by a rather narrow, silvery stripe, and silvery patches at margins; tergum 4 similar but marginal and lateral, silvery stripes and silvery patches at margins more prominent and enclosing an orange stripe; tergum 5 yellowish with some dark markings in centre. Egypt .... *nebulo*

A larger species. Mesonotum light greyish blue with four well-marked dark stripes. Abdomen orange; terga 1 and 2 orange with a narrow, dark median stripe expanding anteriorly to form a dark band; tergum 3 with a broad, dark median stripe widening anteriorly to form a small, dark band, bordered on each side with a wide yellowish white stripe and extreme edges of tergum yellowish white; tergum 4 similar but median, dark stripe very narrow, silvery admedian and marginal stripes broad, and enclosing on each side a dark brown stripe; tergum 5 yellowish white with a rather faint, median dark stripe. Iraq........ *mesopotamiensis*

13. A medium-sized species. Cheeks silvery. Mesonotum dark slate blue with four broad black stripes. Abdomen dark orange; terga 1 and 2 entirely dark orange; tergum 3 with a broad, median dark stripe widening out both anteriorly and posteriorly to form short dark bands, and with well-marked silvery stripes on each side of median stripe and at margins. Egypt .... *xanthomelas*

Larger species. Eyes closely approximated 14

14. Terga 1 and 2 with only anterior half dark brown, a median dark stripe expanding posteriorly to form a small dark band; tergum 3 with a median dark stripe bordered on each side by a broad silvery stripe and silvery patch at margins of tergum, remainder orange; tergum 4 very similar, the median stripe if anything narrower, and posterior margin commonly with dark areas, or narrow dark bands; tergum 5 similar. Vein R_{4+5} usually with a row of well-spaced small bristles on ventral border reaching to bend of M_{1+2}. A large species. China... *convexifrons*

Tergum 1 and 2 dark brown to black........ 15

15. A large species. Abdomen light orange; terga 1 and 2 dark brown except for a small lighter brown patch at postero-lateral border; tergum 3 orange with a broad, median black stripe expanding T-shaped anteriorly to form a partial band, a silvery spot on each side, and a spot at each margin; tergum 4 with a narrow, median dark stripe, and mostly greyish yellow with a narrow admedian orange stripe, posterior border with a narrow dark band which varies in width; tergum 5 mainly greyish yellow with a suggestion of a narrow dark median stripe.. *larvipara*

A slightly smaller species usually with a much *darker* orange (reddish) abdomen, and well-marked bands on terga 3 and 4. Otherwise indistinguishable from *larvipara* and can only be separated with certainty on the characters of the male terminalia .... *autumnalis*

KEY TO THE FEMALES OF THE PALAEARCTIC SPECIES BASED ON EXTERNAL CHARACTERS

1. Mesonotal bristles much reduced, dorsocentrals reduced to a single postsutural bristle and acrostichals entirely wanting. Sternopleurals also wanting. Vertex silvery, about equal to width of an eye; vertical stripe narrow, about one-quarter width of vertex with straight sides; outer vertical hairs small and arranged somewhat irregularly in four and even five rows. Mesonotum greyish white, with four dark shining stripes. Abdomen greyish white, terga 1 and 2 dark brown to black; tergum 3 greyish yellow with a broad, median dark stripe forming a very narrow, anterior dark band and posteriorly a wider dark one, sometimes occupying half the length of the tergum; tergum 4 greyish yellow with a very narrow median dark stripe and two dark, admedian spots. Egypt.............. *albina*

Mesonotal bristles either normal or slightly reduced, especially dorsocentrals and achrostichals. Sternopleurals always present, 1 : 2 2

2. Vein M_{1+2} joining vein R_{4+5}, cell R_5 closed. Vertex wide, almost width of an eye; vertical stripe narrow with straight sides; outer vertical bristles in single row. Thorax metallic green, mesonotal stripes narrow, not well marked except in fresh specimens. Abdomen metallic green; terga 1 and 2 with some orange reflections; tergum 3 and 4 metallic green; tergum 5 metallic green with a large median silvery patch .... *lucidula*

Vein M_{1+2} not joining vein R_{4+5}, cell R_5 narrowly open. Species not metallic 3

3. Mesonotum with *two* black stripes, presutural portion of each Y-forked, the outer branch short. A greyish yellow species. Abdomen greyish yellow to orange (if rubbed); terga 1 and 2 either entirely dark brown or median area light brown .... *sorbens*

Mesonotum with *four* dark stripes.................................. 4

4. Palpi light orange. Proboscis with deep, strongly chitinised, bulbous mentum. A grey species .... *crassirostris*

Palpi dark grey to black. Proboscis normal, mentum not bulbous 5

5. Small to medium-sized grey species with varying amount of metallic sheen.. 6

Medium-sized to large species without any metallic sheen...... 7

6. A small species. Vertex little less than width of an eye; vertical stripe relatively wide with bulging sides; outer vertical bristles in a single row. Mesonotum metallic grey with four rather narrow dark stripes. Abdomen grey with some metallic sheen .... *tempestiva*

A medium-sized species. Vertex greyish white about equal to width of an eye; vertical stripe rather narrow, less than half width of vertex; outer vertical bristles and hairs in four rows. Mesonotum grey with some metallic sheen, the four stripes well marked, the inner pair rather narrow, the outer often rather indistinct. Abdomen grey with some metallic sheen... *vitripennis*

7. Eyes minutely and scantily haired. Abdomen dark brown with bluish grey markings; terga 1 and 2 black with small admedian bluish grey spots; tergum 3 with a broad, median black stripe, large admedian bluish grey spots and smaller silvery lateral spots; tergum 4 similar and tergum 5 yellowish white. Oviparous. Puparium light brown. Widely distributed in North China .... *hervei*

Eyes bare and abdomen not so marked 8

8. Abdomen either entirely orange and without any markings; or either orange, or partly orange with markings.................... 9

Abdomen black with creamy and silvery markings 13

9. Abdomen entirely orange and without any markings. Thorax dark slate blue and stripes somewhat difficult to see. North China .... *ventrosa*

Abdomen either orange, or partly orange, and with markings... 10

10. Abdomen orange and with markings 11

Abdomen only orange at base (partly) and with markings......... 12

11. Vertex and cheeks silvery white; vertex considerably less than width of an eye; vertical stripe a little more than half width of vertex; outer vertical bristles in two rows. Mesonotum bluish white with four broad dark stripes. Abdomen dark orange with silvery markings and, commonly, narrow dark posterior bands to terga. Not a house-fly. Puparium white. Egypt .... *xanthomelas*

Vertex and cheeks creamy white; vertex less than width of an eye; vertical stripe about three-quarters width of vertex; outer vertical bristles in two rows. Mesonotum greyish yellow with four broad, dark stripes. Abdomen light orange with dark stripes and silvery markings. A house fly. Puparium brown. Egypt .... *nebulo*

12. Vertex almost equal to width of an eye; vertical stripe very wide about three-quarters width of vertex and with bulging sides; terga 1 and 2 dark orange, remaining terga greyish with dark markings and some blue sheen .... *domestica*

Vertex and vertical stripe the same as in *domestica*. Abdomen more extensively orange, especially terga 1, 2 and 3........... *vicina*

13. Vertical stripe narrow, about one-third the width of vertex; outer vertical bristles in a *single* row. Mesonotum grey with the usual four, somewhat narrow, dark stripes. Abdomen greyish white with black markings; terga 1 and 2 dark brown to black with grey side spots. Puparium white. Iraq......... *mesopotamiensis*

Vertical stripe wider, about or a little less than half the width of vertex; outer vertical bristles in *two* rows..................... 14

14. Vein R_{4+5} usually with a row of small bristles on ventral surface extending almost to bend of vein M_{1+2}. Markings of abdomen very similar to those of *hervei*, but silvery markings more in the nature of stripes, and in addition well-marked, dark, admedian somewhat triangular spots at posterior borders of terga 3 and 4. Larviparous. North, Central and South China .... *convexifrons*

Vein R_{4+5} never with such small bristles on ventral surface extending to bend of vein M_{1+2}.................................. 15

15. Radius at root on upper surface with three to four hairs......... *autumnalis*

Radius at root on upper surface with five to six hairs.............. *larvipara*

SHORT DESCRIPTIONS AND NOTES ON THE SPECIES

As the main object of this revision is to provide a practical guide to the species of use to medical and veterinary officers and economic entomologists, the descriptions are made as short as possible, and only those characters are noted which will enable the worker to identify his species with certainty. Lengthy descriptions would only defeat this object. I have specially avoided using many chaetotactic characters for the simple reason that I know from long practical experience that they are not only tiresome to use and, therefore, deter many from trying to identify their species, but they are also very unreliable and their value has been much overstated. The identification of the species depends almost entirely on colouration characters, and these, together with such other characters as the width of the vertex in relation to that of an eye, the width and character of the vertical stripe, the arrangement of the outer vertical bristles and hairs, the vestiture of the eyes, the colour of the palps, the structure of the mentum, the presence or absence of the dorsocentral and acrostichal bristles and the sternopleurals, the presence of hairs on the squama, and lastly the presence or absence of bristles and hairs on the ventral surface of vein R_{4+5}, will be found to be most reliable for this purpose. Occasionally atypical specimens will be found which it is almost impossible to name. I would strongly recommend the beginner not to attempt to identify greasy specimens, and especially flies which have not hardened before being pinned. If in doubt, and a male is available, the terminalia should be prepared as noted above, and the mounted specimens compared with the illustrations accompanying this series of papers.

An asterisk accompanying the name of a species means that I have examined the type. A question mark indicates that there is still some doubt as to the identity of the species. As the male terminalia of some of the species of this region have already been illustrated in the first paper (1932), they will not be reproduced again here, and the reader is referred to that paper ; the references to these illustrations have the letter P and the numeral 1 before them, which mean Part I (1932).

(*To be continued*)

THE RELATION OF POLYMORPHIC TRYPANOSOMES, DEVELOPING IN THE GUT OF GLOSSINA, TO THE PERITROPHIC MEMBRANE*

BY

WARRINGTON YORKE,

FREDERICK MURGATROYD

AND

FRANK HAWKING

(*Received for publication* 31 *May*, 1933)

PLATES II AND III.

In the original descriptions by Bruce, Robertson and others of the development of polymorphic trypanosomes in the gut of Glossina, it was assumed that development occurred in the lumen of the gut, and no note was made as to the position of the trypanosomes in relation to the peritrophic membrane. More recent work has shown that this structure, of which the origin and position have been well described by Wigglesworth (1929), plays an important part in determining the position of various flagellate infections. Johnson *et al.* (1928) showed that the gut development of *T. congolense* occurred in the space between the membrane and the gut wall, and Hoare (1931*b*) found that the same was true of the later stages in the development of *T. grayi*. Finally, Taylor (1932) has demonstrated that when *T. gambiense* develops in *G. tachinoides* the trypanosomes multiply almost exclusively in this space external to the peritrophic membrane. The object of the present paper is to record some observations, made during experiments on the transmission of a tryparsamide-resistant strain of *T. brucei* through *G. morsitans* and *G. palpalis*, which confirm and amplify the account given by Taylor.

During the course of our experiments, which have been described elsewhere (Yorke, Murgatroyd and Hawking, 1933), a considerable number of infected Glossina were dissected and especial attention was paid to the position of the trypanosomes. In flies dissected one to four days after an infective feed, trypanosomes could be found in the midgut, mainly in its posterior half, multiplying in the lumen of the gut *inside* the peritrophic membrane. Presumably this multiplication represents the first stage of the establishment of the parasite in its invertebrate host. But only a certain proportion of these flies develop a permanent infection. Although no trypanosomes were seen in the space outside the membrane during examination of the fresh specimen at this stage, some forms may be present there even at this early date, as was discovered by embedding and sectioning one of these infected guts. Apparently the trypanosomes reach this space by passing round the free end of the membrane at the lower end of the alimentary canal. After the first feed on a clean animal, trypanosomes are no longer found *inside* the membrane, but they may be found in great numbers multiplying in the space *outside* the membrane. Eventually they pass forward along this space and reach the proventriculus, as described by Taylor.

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* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

A number of the flies, which were suspected of being infective, were examined by drawing out one of the salivary glands through the neck. The gland was examined and, if trypanosomes were present, the whole fly was fixed in Bouin and subsequently embedded in paraffin, according to the technique of Hoare (1931*a*). In this way sections were obtained with a minimum of disturbance of the internal organs. The sections were stained with Mayer's haemalum. The accompanying drawings (Pls. II and III) illustrate the more important points.

The first drawing is taken from the midgut in the region described by Wigglesworth as the anterior segment. At this level the ingested blood has been concentrated by the absorption of water, but the red corpuscles are still intact and no haematin has been liberated. The figure shows masses of trypanosomes lying perpendicularly to the wall of the gut in the space between the wall and the peritrophic membrane. Trypanosomes are also present (external to the membrane) in a fold of the membrane invaginated into the lumen of the gut. In none of the sections of the midgut (excluding the proventiculus) were trypanosomes found *inside* the peritrophic membrane in flies which had had several feeds on a clean animal. The distribution of the flagellates along the length of the gut probably varies according to the time since the infection commenced. In these flies, two to four weeks after the infecting feed, trypanosomes were most numerous in the posterior part of the anterior segment and the anterior part of the middle segment (as defined by Wigglesworth). Small numbers were present along the rest of the midgut, except in the posterior part just above the entry of the Malpighian tubules, in which region no trypanosomes were seen at this later stage of the infection. No trypanosomes were found in the hindgut or Malpighian tubules.

The next figure (fig. 2) shows a transverse section of the proventriculus. According to the account given by Wigglesworth, this organ acts, firstly, as a sphincter between the foregut (oesophagus) and the midgut, and, secondly, as the source from which the peritrophic membrane is derived. The cells of the annular pad (*ep.*) secrete a fluid, which is squeezed out through the narrow cleft between the wall of the proventriculus and the invaginated portion of the foregut (*in. fg.*), and is thus moulded to form a thin membrane. Trypanosomes (t_1) can be seen in the extraperiptrophic space, which is bounded on the one side by the peritrophic membrane and on the other by the walls of the proventriculus and midgut. They (t_2) are also to be seen in the space bounded by the secretory epithelial cells (*ep.*) and the invaginated portion of the foregut (*in. fg.*) ; in this region the parasites can pass through the fluid secretion of the epithelial cells to reach the lumen of the proventriculus.

Fig. 3 illustrates a longitudinal section of the proventriculus showing the forward path of the developing trypanosomes from the extraperitrophic space (t_1), through the fluid secretion of the annular pad (t_2), into the lumen of the proventriculus and oesophagus (t_3).

Fig. 4 shows part of a tangential section taken through the anterior edge of the proventriculus (from the same specimen as fig. 2). A large mass of trypanosomes is seen in the space between the invaginated foregut and the annular pad of epithelial cells, but these latter do not appear in the part of the section here shown. The last drawing (fig. 5) shows a section through one of the infected salivary glands and requires no comment.

SUMMARY

During the course of experiments on the transmission of a tryparsamide-resistant strain of *T. brucei* through *G. morsitans* and *G. palpalis*, observations were made regarding the position of the developing trypanosomes in relation to the peritrophic membrane of the midgut and proventriculus. After the first few days of the infection trypanosomes were found to be limited to the space outside the peritrophic membrane—between the membrane and the gut wall. As the infection develops, the trypanosomes gradually extend forwards along this extraperitrophic space as far as the proventriculus, where they pass through the fluid secretion of the annular pad (from which the membrane is formed) and so reach the lumen of the proventriculus and oesophagus. Drawings of sections are given, demonstrating this development of the infection. These observations confirm those previously published by Taylor.

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PLATE II

EXPLANATION OF PLATE II

Fig. 1. LONGITUDINAL SECTION OF WALL OF MIDGUT (Anterior Segment). (× 460.) This section shows the masses of trypanosomes limited to the space between the peritrophic membrane and the epithelial lining of the gut, and to the space formed by the invaginations of the membrane into the lumen of the gut.

ep. = epithelial cells constituting the wall of the midgut.
p.m. = peritrophic membrane.
p.m.$_1$ = peritrophic membrane, invaginated into the lumen of midgut, containing numerous trypanosomes.
r.b.c. = undigested red blood corpuscles in lumen of midgut.
t. = trypanosomes in the space between the peritrophic membrane and the gut wall.

Fig. 2. TRANSVERSE SECTION OF PROVENTRICULUS. (× 275.) This section shows the trypanosomes in the extraperitrophic space of the midgut and in the space where the peritrophic membrane is formed.

ep. = annular pad of large epithelial cells which secrete the peritrophic membrane.
in.fg. = invaginated portion of foregut.
mg. = commencement of midgut.
oes. = oesophagus (foregut).
p.m. = peritrophic membrane.
t_1 = trypanosomes outside the peritrophic membrane.
t_2 = trypanosomes in the space where the peritrophic membrane is formed.

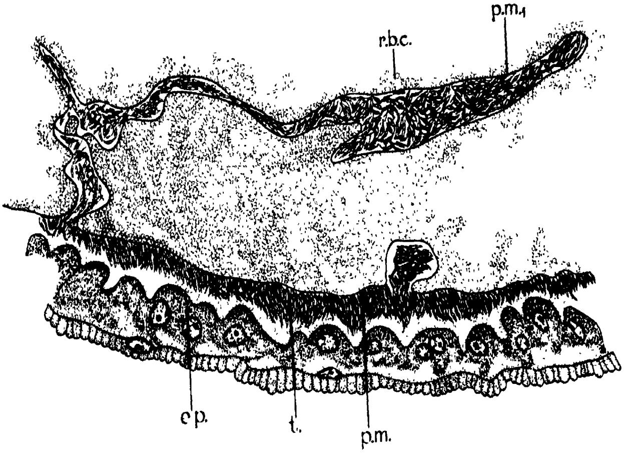


Fig. 1

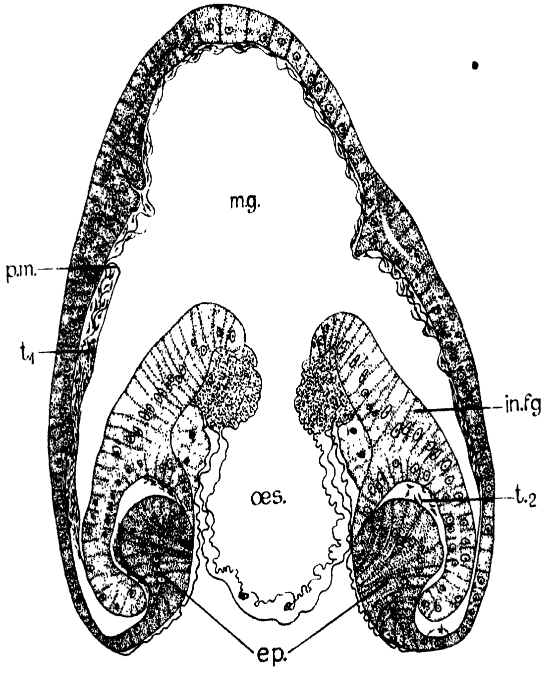


Fig. 2

EXPLANATION OF PLATE III

Fig. 3. Longitudinal Section of Proventriculus. (× 125.) This section shows the forward path of the developing trypanosomes and the manner in which they pass from the space in the midgut outside the peritrophic membrane into the lumen of the proventriculus and thence into the oesophagus.

ep. = annular pad of large epithelial cells, which secrete the peritrophic membrane.
oes.d. = duct of oesophageal diverticulum (sucking stomach).
in.fg. = invaginated portion of foregut.
mg. = commencement of midgut.
oes. = oesophagus.
p.m. = peritrophic membrane.
t_1 = trypanosomes in the space outside the peritrophic membrane at the commencement of the midgut.
t_2 = trypanosomes in space where the peritrophic membrane is formed.
t_3 = trypanosomes in lumen of foregut (oesophagus and duct of oesophageal diverticulum).

Fig. 4. Tangential Section Through Edge of Proventriculus. (× 275.) Showing masses of trypanosomes in the space where the proventriculus is formed.

in.fg. = invaginated portion of foregut.
o.p. = outer wall of proventriculus.
p.m. = peritrophic membrane.
t. = trypanosomes in the space where the peritrophic membrane is formed.

Fig. 5. Transverse Section Through Salivary Gland. (× 460.) Showing numerous trypanosomes attached to the wall of the gland or lying free in the lumen.

PLATE III

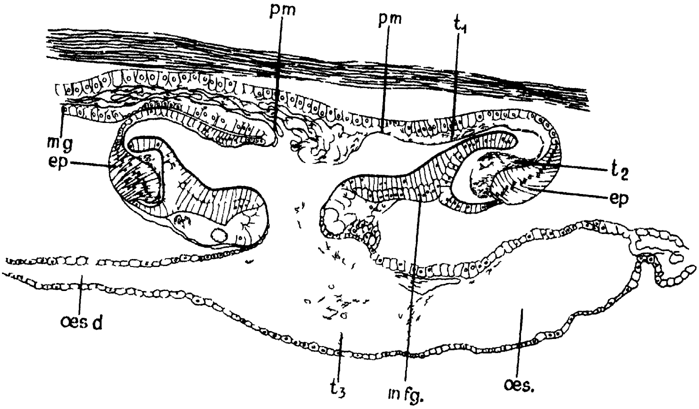


Fig 3

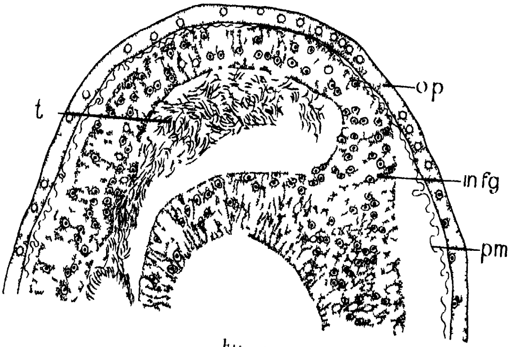
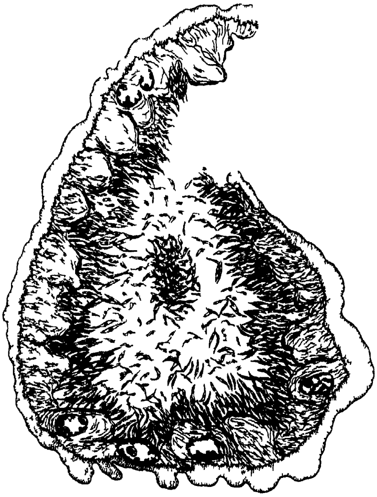


Fig 4



MISCELLANEA

RELATIVE SUSCEPTIBILITY OF THE SEXES OF GLOSSINA TO INFECTION WITH TRYPANOSOMES

Mr. J. S. McDonald, Laboratory Assistant to the Human Trypanosomiasis Institute, has of late been examining my registers wherein are recorded the transmission experiments with tsetse and various polymorphic trypanosomes performed during the last six or seven years. In my paper, published in Vol. XXIV of the 'Annals of Tropical Medicine and Parasitology,' entitled 'On the Susceptibility of the Two Sexes of *G. palpalis* to Infection with *T. gambiense* and *T. rhodesiense*,' the conclusion was reached that there existed no significant difference between the two sexes of *G. palpalis* in their susceptibility to infection with *T. gambiense* or *T. rhodesiense*. Mr. McDonald's examination of the data presented in that paper shows that this conclusion is not justified by the evidence, the reason for this opinion being, in his own words, as follows :—

'A hypothesis that there is no variation in the susceptibility of the sexes to infection requires that there shall be no significant deviation in the observed infections, in either sex, from the common infection rate of the whole population examined.

'The best value to assume for the infection rate common to both sexes is that given by dividing the number of infections observed by the number of flies examined. From this value the number of infections to be expected for each sex can be obtained.

'The population of 24,509 flies contained 1,237 infections, so the common infection rate is expressed as 1,237 ÷ 24,509. The expected infection rates for 12,737 males and 11,722 females are therefore :—

$$\frac{1{,}237 \times 12{,}737}{24{,}509} \quad \text{and} \quad \frac{1{,}237 \times 11{,}722}{24{,}509}$$

643 and 594 respectively.

	Infected	Negative	Totals
Males	581	12,156	12,737
Females ...	656	11,116	11,772
Totals	1,237	23,272	24,509

'Examined by the χ^2 formula, $\chi^2 = \left(\frac{x-m}{m}\right)^2$, which measures in this case the probability that equal or greater differences than those observed would be likely to occur if there was no variation of susceptibility between the sexes, the data presented in the paper give $\chi^2 = 13$ and $P = 0{\cdot}01$, which is a highly significant value indicating that such variations could occur by chance only once in considerably over 100 trials.

'It appears therefore that, at all events under laboratory conditions, the female of *G. palpalis* is more susceptible to infection with *T. gambiense* or with *T. rhodesiense* than is the male.'

'On reflection, there are two observations which may have a bearing on this conclusion, namely, that the female flies on the whole feed somewhat more readily than the males, and that they tend to survive better the exigencies of prolonged service in captivity.

H. LYNDHURST DUKE.

7 *March*, 1933.

THE IDENTITY OF *CULEX AEGYPTI* L.

In the last issue of the 'Annals of Tropical Medicine and Parasitology,' Professor W. S. Patton published a short article under the above heading which calls for some reply on account of the importance of reaching agreement, if possible, upon the scientific name to be applied to the yellow-fever mosquito.

Professor Patton is not correct in stating that in 1932 I changed the name from *fasciata* to *aegypti* without giving my reasons for doing so. The change was first proposed in print by Dyar in 1920, and the name *aegypti* has since then been used by nearly all American writers as well as by many in Europe and elsewhere. I gave a reference to Dyar's paper in my catalogue, and also stated (p. 2) that I 'accepted the view that the yellow-fever mosquito should be known as *Aëdes aegypti* L., as in spite of some discrepancies it seems hardly possible that Linnaeus could have had any other species before him.'

Professor Patton quotes an opinion of the late Dr. L. H. Gough that *C. aegypti* was really the species now commonly known as *Aëdes caspius*, and attempts to support Gough's opinion by reference to the original descriptions of *C. aegypti*. When I first re-published the description of *C. aegypti* in 1911, I also thought it *might* refer to *A. caspius*, chiefly because this is one of the commonest Egyptian species and because Linnaeus referred to white rings on the legs 'circa articulationes et in articulis.' If I remember correctly, I made this suggestion to Gough and he adopted it. However, a more careful reading of the description convinced me that it was more likely that Linnaeus was describing a species of *Stegomyia*, and I still think the description indicates this as clearly as one could expect; I will follow Professor Patton's example and consider this description phrase by phrase.

The opening words, '*Culex aegypti* articulationibus candidis,' show that Linnaeus picked out as the most salient feature of the species the white-ringed legs, which are, in fact, so conspicuous in a *Stegomyia*. The word *candidis* ('glistening-white') could not be applied to the pale tarsal rings of *A. caspius*, which are at most dull creamy-white and are certainly not the most striking feature of the insect.

'*Magnitudo Culicis vulgaris* Linn. *Syst. N. I.*' This tells us nothing definite.

'*Color ex fusco canus.*' I cannot agree with Professor Patton in attaching special importance to this phrase, nor can I agree with the translation of it which he quotes. My dictionary gives us the meaning of *fuscus*, 'dark, black' and of *canus*, 'whitish-grey, grey.' The meaning of the phrase is somewhat obscure, but I should freely translate it 'hoary greyish-black.' If it really meant 'tawny shading into grey,' it might indeed be held applicable to *A. caspius*, but this translation seems too great a distortion of the actual words.

'*Crura cana cum annulis candidis, parvis, circa articulationes et in articulis.*' This presumably refers to the white rings of the hind tarsi. In *A. caspius* the pale tarsal rings spread over both sides of the joints; in the yellow-fever mosquito they are at the base of the joints only. The words 'circa articulationes' might be taken to indicate the former, but it seems doubtful if Linnaeus would have noted this distinction in the position of the rings, and I should translate the phrase 'legs grey with small pure white rings at the joints and on (each) segment.'

'*Puncta candida ad marginem dorsi in corpore sub alis utrinque, plura, longitudinaliter sita.*' Professor Patton thinks this refers to markings on the sides of the thorax, but in this he is almost certainly mistaken. It appears to me fairly obvious that Linnaeus was referring to the row of spots on the *abdominal tergites*, which are conspicuous in side view even when the wings are folded over the back ('sub alis' suggests that the wings in Linnaeus' specimen were so placed). The words '*puncta candida*' again indicate the silvery-white spots of the yellow-fever mosquito rather than the less definite and dull white markings of *A. caspius*.

'*Annulus candidus ad basin thoracis, inter illum et corpus.*' I have no doubt that this refers to the silvery-white scutellum of a *Stegomyia*, as Professor Patton himself suggests. With the wings folded over the back, the dorsal abdominal markings would not be visible, and the scutellum would conspicuously separate the thorax and abdomen. If Linnaeus had had before him a specimen of *A. caspius* with the first abdominal segment visible, he would certainly have described other dorsal abdominal markings.

'*Linea candida perpendicularis juxta oculos, utrinque una parva.*'

This would apply very well to the line of silvery-white scales on each side of the head, perpendicular to the eyes, in the yellow-fever mosquito; alternatively, it might possibly apply to the silvery-white scales on the basal antennal segments, or to the two patches on the clypeus, or even possibly to the white tips of the palpi, but it does *not* apply to the colour of the head-scales in *A. caspius*.

I therefore contend that there is nothing in the description which 'definitely rules out *S. fasciata*,' but that, on the other hand, there are several points which definitely indicate some species of *Stegomyia*. It might, of course, be held that the description would apply equally to another *Stegomyia*, but so far as we know the yellow-fever mosquito is the only species of the sub-genus occurring in Egypt; hence I conclude that 'Linnaeus could hardly have had any other species before him.' The omission of any reference to the crescentic white marks on the thorax is easily explained by supposing that the specimen was pinned (as usual at that period) with a large pin which obscured the thoracic markings.

Professor Patton suggests that 'there seems no reason to suppose that the name-hunter may not yet find an even older one than that of *aegypti*.' As zoological nomenclature commences from the year 1758, and the name *aegypti* was introduced only four years later, it is remotely improbable that any such discovery will ever be made.

Professor Patton wishes to revert to the use of the name *fasciata*, but this course is open to two serious objections. In the first place, in most of the literature published in the last fifteen or twenty years the yellow-fever mosquito is referred to either as *Aëdes aegypti* or as *Aëdes argenteus*; there would therefore be no gain on the score of convenience in dropping both these names in favour of *fasciata*. In the second place, according to a strict application of the code of zoological nomenclature, the name *Culex fasciatus* Fabricius is invalidated by the earlier *Culex fasciatus* Müller, even though the former is now transferred to the genus *Aëdes* and the latter is probably a synonym of *Culex pipiens*. It would of course be possible to obtain a ruling from the nomenclature committee setting aside the rule in this particular case and treating *Aëdes (Stegomyia) fasciata* as a *nomen conservandum*. Until 1919 I was in favour of this course, but was strongly opposed by Dyar and others; definite action has never been taken on these lines and I believe it is now too late. In adopting *aegypti* in place of *argenteus* in the 'Genera Insectorum' (an action which I only took after considerable correspondence with other workers), I hoped that it might be possible to arrive at uniformity for the future.

F. W. EDWARDS.

14 *April*, 1933.

In the absence of the specimen from which Linné drew up his description of *C. aegypti*, Dr. Edwards's interpretation of it leaves one as much in doubt as does the unbiased translation of it given me by Dr. Mountford, Professor of Latin in this University, and my interpretation of *C. aegypti* from it. As this point can never be settled with certainty, no matter what ingenuity may be displayed in picturing Linné's specimen of *C. aegypti*, I consider it is best to follow the spirit rather than the letter of the law of priority and to drop the name *aegypti*.

W. S. PATTON.

16 *May*, 1933.

Volume XXVII October 21st, 1933 No. 3

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(2) Two courses for the Diploma in Tropical Hygiene, commencing on the 11th January, 1934, and the 26th April, 1934. The D.T.H. examinations are held in March and June.

(3) Two courses in Veterinary Parasitology, commencing on the 2nd October, 1933, and the 3rd January, 1934.

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THE CHARACTERISTICS OF SOME NIGERIAN STRAINS OF THE POLYMORPHIC TRYPANOSOMES

BY

H. M. O. LESTER

CORRIGENDA

' Natural malaria infection of house-frequenting *Anopheles* mosquitoes in Uganda,' by E. G. Gibbins, Vol. XXVI, No. 3, October, 1932.

Page 243, line 32, ' less heavily infected areas ' should read ' less heavily infested areas.'

Page 249, line 8, ' (*d*) Climate Factors ' should read '(*d*) Climatic Factors.'

Page 261, line 1, ' out of 552 and 553 glands ' should read ' out of 552 stomachs and 553 glands.'

Page 261, line 12, ' vicinity of the River ' should read ' vicinity of the River Nile.'

' The domestic *Anopheles* mosquitoes of Uganda,' by E. G Gibbins, Vol. XXVII, No. 1, April, 1933.

Page 25, line 5, ' prevalence of males does appear to be of much significance ' should read ' prevalence of males does not appear to be of much significance.'

' On *Anopheles funestus* and its allies in the Transvaal,' by Botha De Meillon, Vol. XXVII, No. 1, April, 1933.

Page 91, column 18, ' *Cinctus* ' should read ' *Cinereus.*'

' Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. I : A review of existing knowledge on this subject, with some general observations,' by H. Lyndhurst Duke, Vol. XXVII, No. 1, April, 1933.

Page 113, horizontal column 5, ' 70 of the strains were non-transmissible ' should read ' several of the strains were non-transmissible.'

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

THE CHARACTERISTICS OF SOME NIGERIAN STRAINS OF THE POLYMORPHIC TRYPANOSOMES

BY

H. M. O. LESTER

(*From the Tsetse Investigation Laboratory, Northern Nigeria*)

(*Received for publication* 18 *May*, 1933)

It has been generally believed that sleeping sickness in West Africa is due invariably to *T. gambiense* and that the more acute forms of the disease ascribed to *T. rhodesiense* do not occur. Macfie (1913) described a very mild type which he met with in the Eket region of Southern Nigeria. The morphology of the causative trypanosome, together with its low virulence to human beings, led him to think that it must be a new species, and he proposed calling it *Trypanosoma nigeriense.* This view was not generally accepted and *T. nigeriense* is considered to be merely an almost avirulent strain of *T. gambiense.*

In Northern Nigeria there are considerable differences in the types of human trypanosomiasis which are encountered. Throughout the southern part of the country, where *G. palpalis* is the chief vector, the disease considered as a whole has a comparatively mild endemic form, although there are occasional localised epidemics of a more virulent nature. Further north, where *G. tachinoides* is the chief vector, there are large epidemic areas in which the disease seems to be more virulent. These differences are striking, and as a general rule it can be said that the farther one goes south the less virulent does the disease become.

From time to time sleeping sickness medical officers have reported cases resistant to treatment with tryparsamide. Such cases have occurred both in the endemic and epidemic areas. The existence of these tryparsamide-resistant strains was rather brought home to us by the fact that in 1929 G. B. B., a European member of the Tsetse Investigation Staff, contracted sleeping sickness while working in the field. This case was peculiar in that, while undergoing treatment, his blood was found to be swarming with trypanosomes, even though he had received repeated injections of tryparsamide. Eventually he was given a full course of ' Bayer 205 ' and had an uneventful recovery.

Unfortunately, at that time it was not possible for us to isolate the strain for investigation. On looking up old laboratory records, it was apparent that there were great differences in the way strains isolated from local sleeping sickness patients behaved in small laboratory animals. Many strains failed to take in guinea-pigs, and could be made to infect the smaller laboratory animals only after preliminary direct passage through monkeys. It was common to find that in chronically infected guinea-pigs, after some months there appeared to be a sudden change in the character of the strains. Trypanosomes became very numerous in the peripheral blood, and in subsequent direct passages strains seemed to have an exalted virulence. Occasionally strains have been isolated that had a high initial virulence to small laboratory animals. In one such case, the inoculation of a few drops of gland juice from a sleeping sickness patient into a white mouse produced a massive infection in seven days.

In 1929, Dr. J. C. Paisley isolated a strain (' Sherifuri K ') from an untreated local sleeping sickness case. A few drops of gland juice injected into a guinea-pig produced an infection in 12 days and trypanosomes were numerous in the blood on the fourteenth day. Stained blood films from a series of small laboratory animals showed the presence of numerous posterior nuclear forms.

In view of these findings and in the hope of throwing some light on the epidemiology of the disease, it was decided to attempt a thorough laboratory investigation into the characteristics of a number of trypanosome strains isolated from patients in contrasting areas.

ORIGIN OF STRAINS

The first series of strains was obtained from untreated patients attending Gadau dispensary. These were people who had come from the big epidemic area to the north of Gadau. The infected region, comprising parts of Hadejia and Katagum Emirates and Bedde Emirate, is situated in zone lat. 12° to 13°, long. 10° to 11° E. It is traversed by the Hadejia and Katagum rivers and their tributaries. The majority of the country is covered with a light savannah forest, with areas of thick bush adjacent to the rivers. In the dry season the whole area is very arid, and the rivers, with their wide sandy beds, dry up, leaving a series of pools. In the wet season, on the other hand, the rivers tend to flood their banks, and the whole of the northern part of the area consists of a series of great swamps, separated by sand ridges on which the inhabitants have their villages and farms. In spite of these disadvantages the area supports a large population. In the neighbourhood of the rivers game is comparatively numerous and some localities are infested with *G. morsitans*. *G. tachinoides* is much more wide-spread, and during the rains this species encroaches on to the farms and even into the villages. The comparatively large farmed areas round villages help to protect the people from tsetse. The game and with it *G. morsitans* tend to be localised to a great extent, so that the ordinary farmer does not come into close contact with this fly, although there are always numbers of the people working throughout the bush, collecting honey, firewood, edible fruits, etc.

G. tachinoides is obviously the chief vector of human trypanosomiasis in the locality. About 10 per cent. of the population is infected with the disease, the infection rate being highest in the neighbourhood of the rivers and tailing off as the drier zones are approached. In some villages infection rates of 60 to 70 per cent. have been recorded. During the last two or three years, about 10,000 cases of sleeping sickness have been treated from the area. Of these, about 1,500 came in voluntarily to seek treatment, while the remainder have been met with while carrying out mass examinations in the field. Most of the cases that come in voluntarily complain of sleepiness and show nervous manifestations. They usually give a history of about nine months to a year. The majority of them have enlarged cervical glands, though cases are met with where diagnosis can only be made by examination of thick blood films. Numbers complain of vague toxic symptoms and show no clinical signs of central nervous system involvement ; as they have no enlarged cervical glands, diagnosis is not always easy. It is noteworthy that, of such cases attending at Gadau, lumbar puncture almost always shows some degree of central nervous system involvement. A certain amount of generalised oedema is common, though this is rarely so well marked as to be the chief symptom. It is rare to meet with cases in which trypanosomes are numerous in the peripheral blood. In the field, the majority of patients do not show characteristic nervous symptoms ; quite often they have few symptoms of any kind. There is evidence to show that a number of these cases die before they reach the later stages of the disease. In one survey, 80 out of 2,700 of such people died in an average interval of three weeks between examination and treatment. Dr. J. C. Paisley carried out a mass examination of one particular village in 1930 and found the majority of inhabitants to be infected. The people refused treatment on the plea that they were not ill. Six months later numbers of them came in voluntarily to Gadau and were then found to be in the last stages of the disease. Enquiry elucidated the fact that many of the others had died in the interval. A short time later the whole village was deserted. Apparently the epidemic is of a recent origin and was spreading rapidly in an area which had previously been endemic.

The other group of strains was derived from Ayu district, Jemaa division of Plateau Province. Ayu is a small district situated about 300 miles south-east of Gadau. The country is for the most part open and hilly, with patches of thick bush, containing *G. palpalis*, in the hollows. Game is not plentiful ; there are fair numbers of cob and duiker, but little else. As far as is known, there is no belt of *G. morsitans* within many miles of the area, the vector of sleeping sickness being *G. palpalis.*

Dr. C. W. Hope Gill carried out a mass examination of the district in April, 1931 : out of a population of 3,444, he found 172 cases of sleeping sickness—an infection rate of 5 per cent. The disease appeared to be clinically of a very mild type and individual cases gave histories of long duration without showing any obvious signs of nervous involvement. Out of 138 lumbar punctures made on known positive cases, 81 (56 per cent.) gave increased white cell counts. When the mild character clinically of the cases is considered, it is perhaps surprising that such a large proportion of them showed signs of infection of the nervous system, though in the majority the increase in the cell count was comparatively small. During the period of two and a half months which elapsed before these patients could be treated, there were seven deaths out of the 172 cases diagnosed. At the time of the survey, several *Macacus rhesus* monkeys were injected with blood from untreated patients and were sent back to the Gadau laboratories. These Gadau and Ayu strains were compared with four *T. brucei* strains isolated in the Gadau district and with two old laboratory strains of *T. gambiense* and *T. rhodesiense* which had been kept for many years in small animals in London.

EXPERIMENTAL

At first it was decided to investigate the following characteristics : virulence in small laboratory animals, morphology, reaction to tryparsamide and reaction to the adhesion test. The publication by Yorke, Adams and Murgatroyd (1930) of their work on the action of normal human serum *in vitro* led to this programme being modified to include the use of their technique for the investigation of the action of human serum *in vitro*. Mention has already been made of the virulent strain showing numerous posterior nuclear forms isolated by Dr. Paisley in 1929. Yorke and his co-workers tested this strain ('Sherifuri K') against normal human serum *in vitro*. They found that trypanosomes were destroyed in six hours and concluded that 'Sherifuri K' was a strain of *T. rhodesiense* which was in process of losing its resistance to human serum. Previous work (Johnson and Lester, 1929) led the writer to expect that interesting results might be obtained from the adhesion test. A number of such tests carried out with the Gadau strains gave rather anomalous results. When freshly isolated, the strains gave strongly positive results against sera from sleeping sickness cases and against sera from experimental animals infected with other human strains and treated with tryparsamide; no positive results were obtained with sera from treated *brucei* animals. When once the strains had been maintained for several months, similar clear-cut results could not be obtained. Sometimes the test would give a positive result only when trypanosomes were put up against sera from animals infected with the same strain, whereas at other times slight positive results would be obtained with sera, both from sleeping sickness patients and from *brucei* animals. On account of these inconsistencies, work with the adhesion test was not continued.

HISTORY OF STRAINS

The Gadau and Ayu strains were isolated by the intra-peritoneal injection of 5 c.c. of blood from untreated sleeping sickness patients into *Macacus rhesus* monkeys. From the monkeys the strains were passed into guinea-pigs, and during the course of these investigations were kept in series of guinea-pigs by constant syringe passage. Some 15 human strains and 4 of *T. brucei* were used.

Gadau Strains. - Strains Gadau 1 to 4 were isolated in May, 1930. Gadau 5 was a stock laboratory strain isolated in July, 1929. Gadau 6 was isolated in October, 1930, and Gadau 7 in January, 1932.

Ayu Strains.-- The monkeys inoculated by Dr. C. W. Hope Gill in April, 1931, were sent back to Gadau and were used by Taylor (1932) for a series of cyclical transmissions in guinea-pigs, after which they were handed over to Dr. R. D. Mackenzie and the writer. Ayu 5, 7, 8 and 11 had been transmitted through *G. tachinoides*, while Ayu 6 had undergone one transmission by *G. morsitans.*

London Strains.—The London strains of *T. gambiense* and *T. rhodesiense* were obtained by Dr. R. D. Mackenzie through the kindness of Prof. J. G. Thomson, of the London School of Hygiene and Tropical Medicine. The *T. gambiense* strain had been isolated in November, 1921, from a patient from Nigeria, while the *T. rhodesiense* strain had been isolated in January, 1923, from a patient from the Luanga Valley. Both strains had been kept in small laboratory animals and had not undergone cyclical transmission since the time of their isolation from man. In 1931, the strains were taken out to Nigeria in guinea-pigs and were maintained afterwards in guinea-pigs and white rats.

Brucei Strains.—*Brucei* 1, a stock laboratory strain, was isolated from wild *G. morsitans* in 1928, and had been maintained in a series of dogs and monkeys.

Brucei 2 was isolated from wild *G. morsitans* in May, 1930, and was first maintained in dogs and then in guinea-pigs.

Brucei 3 was obtained from a naturally infected dog in November, 1932, while *brucei* 4 was isolated from wild *G. morsitans* at the same time. Both strains had been maintained in guinea-pigs.

VIRULENCE IN SMALL LABORATORY ANIMALS

The virulence of the strains has been worked out chiefly in guinea-pigs. In assessing virulence, the factors to be noted are (*a*) the incubation period, (*b*) the character of the disease, as indicated by the numbers of trypanosomes in the peripheral blood, and (*c*) the duration of the disease. Kligler and Rabinowitch (1927) have shown that the incubation period varies according to the number of trypanosomes inoculated.

In the Gadau laboratories, it has been possible to vary the incubation period of a strain of *T. brucei* in white rats from one to ten days, according to the number of trypanosomes injected. Apparently, when comparatively few trypanosomes are injected into an animal, the resistance of the host may be able to control the disease to some extent; on the other hand, the injection of many hundreds of millions of trypanosomes may overcome completely the natural resistance at the start and so profoundly effect the whole course of the disease. This seems to be a matter of considerable importance, as it is obvious that when an infected tsetse-fly bites an animal only a comparatively small number of trypanosomes can be injected. In the same way, the inoculation of a few c.cs. of blood from a patient suffering from chronic sleeping sickness entails the injection of a comparatively small number of trypanosomes. On the other hand, with direct transmission in laboratory animals, it is easy to choose just the opportune moment, when trypanosomes are comparatively numerous in the blood, for passing on the strain. In this way the infecting doses tend to get bigger and bigger, and this may play a considerable part in producing the increase in virulence which occurs so commonly with laboratory strains.

The following is a summary of the observations made :—

Gadau strains 1 to 5 and 7 behaved in a way typical of *T. gambiense*: the first few guinea-pigs inoculated showed a very chronic infection and lived from 100 to 200 days. Of these strains, Gadau 4 seemed to have the highest virulence, trypanosomes starting to become numerous in the blood of the first guinea-pig a month after inoculation from the monkey. Gadau 3 had a very low virulence and it was five months before trypanosomes became numerous in a guinea-pig; with this particular strain it was not possible to lower the incubation period to less than ten days, however big the infecting dose, while with the other strains incubation periods of two and three days could be obtained by judicious dosage. Strain Gadau 6 was very virulent from the start. Trypanosomes showed up numerous in a monkey 14 days after its inoculation from man. It was sub-inoculated into a guinea-pig, and trypanosomes became fairly numerous 6 days later, the animal dying on the eighth day. Sub-inoculated into rats, swarming infections were produced on the fourth day. After a few direct passages, this strain killed guinea-pigs in 7 to 10 days and white rats in even less time.

Ayu strains 5, 7, 8, 9 and 11 were obtained from chronically infected guinea-pigs left after *G. tachinoides* transmission experiments. They were all comparatively avirulent, and their behaviour in guinea-pigs was very similar to that of Gadau strains 1 to 5 and 7. Ayu 6 was obtained from a guinea-pig which had been used for a

G. morsitans transmission experiment. It was found to be very virulent and was similar in this respect to Gadau 6.

London Strains.—Both these old laboratory strains were very virulent and killed both guinea-pigs and white rats in a few days.

Brucei Strains.—Strain *brucei* 2 was not particularly virulent when first isolated, but after a few direct passages its virulence became very much exalted. *Brucei* 1, 3 and 4 were all virulent to small laboratory animals, but if anything they were less so to guinea-pigs than were Gadau 6, Ayu 6 and the old London strains.

THE OCCURRENCE OF POSTERIOR NUCLEAR FORMS

Lavier (1927) and Kleine (1927) both demonstrated the presence of posterior nuclear forms in *T. gambiense* infections. Lavier, working with guinea-pigs, found occasional posterior nuclear forms in each of the seven strains of *T. gambiense* which he examined. He noted that posterior displacement of the nucleus was less common and not so well marked as in *T. rhodesiense* infection ; in particular, he never found the nucleus posterior to the blepharoplast.

The writer has been able to confirm these results, in that posterior nuclear forms have been found in all the Nigerian strains in which an adequate number of short forms was counted. The standard of posterior displacement adopted was that the nucleus must lie completely within the posterior third of the trypanosome. In examining slides, for the first 200 to 300 trypanosomes, both long and short forms were counted so as to obtain the percentage of short forms. After this, in order to save time, only short forms were noted and the number of posterior forms worked per cent. of short forms.

Occasional posterior nuclear forms only were found in the following strains : Gadau 1, 2, 4 and 7 and Ayu 7, 8, 9 and 11. Posterior nuclear forms were found to be comparatively numerous in Gadau 3, 5 and 6, Ayu 5 and 6 and in the *brucei* strains. Counts were not made for the old laboratory strains. Within the individual strains great variations were noted at different times.

Strain Gadau 3.—A number of guinea-pigs were examined, and about 4,000 short forms counted. In individual animals posterior nuclear forms varied from 0 to 15 per cent. of all short forms. With several animals percentages of 10 to 15 were quite common. The following Table gives examples of counts made from time to time on two of the guinea-pigs.

TABLE I

Showing variations in the percentage of posterior nuclear forms in the guinea-pigs infected with Gadau 3

No. of animal	Date	No. of short forms per cent. of total tryps.	Total no. of short forms counted	No. of posterior nuclear forms	No. of posterior nuclear forms per cent. of total short forms
Guinea-pig 19	8/8/30	93·0	202	0	0
	17/8/30	17·1	184	0	0
	3/9/30	26·4	157	4	2·5
Guinea-pig 50	7/11/30	20·1	226	34	15
	13/11/30	51·0	225	4	1·8
	19/12/30	25·2	344	30	8·7
	30/12/30	49·7	266	16	6·0

Gadau 5. -At the time that this strain was examined it was already 18 months old. Posterior nuclear forms were found up to 4·5 per cent. In one guinea-pig one form was noted which had its nucleus posterior to the blepharoplast.

Gadau 6.– This strain, together with the other virulent strains, killed off laboratory animals with great rapidity. So much was this so that the Gadau stock of experimental animals was insufficient for their maintenance for considerable lengths of time, and when once the main tests were completed, the strains had to be discarded. Unfortunately, the series of guinea-pig blood films which had been saved for future examination showed a great proportion of long slender forms, and short forms were rare. In a mouse short forms were more numerous, and 23 per cent. of these were found to have posterior displacement of the nucleus.

Ayu 5.—Numbers of both guinea-pigs and mice were examined, and posterior nuclear forms found to be comparatively common. Percentages of from 0 to 17 being obtained in individual animals.

Ayu 6.—There was a great proportion of long slender forms in the slides saved for examination. In mice, posterior nuclear forms were numerous, percentages up to 29·4 being obtained.

REACTION TO TRYPARSAMIDE

The reaction of all strains to tryparsamide was tested in guinea-pigs. These animals were found to be the most suitable, in that heavy infections could be obtained fairly easily, whereas both white rats and white mice seemed to be little susceptible to some of the less virulent strains. The number of experimental animals required for an exhaustive testing of a considerable number of strains is very large, and guinea-pigs were the only animals that we possessed in anything like sufficient numbers for the purpose. As it was, the earlier tests had to be somewhat incomplete. Where possible, 5 animals were treated at a time and were given intraperitoneal injections of measured doses of tryparsamide made up in 2 c.c. of water. As far as it could be arranged, animals were not treated until their infections were 2 to 4 weeks old, with trypanosomes moderately numerous in the peripheral blood. It is obvious that this could hardly be done with the more virulent strains.

The object of the tests was to determine the dose of tryparsamide in grammes per body weight of guinea-pig which would sterilise the peripheral blood. Yorke, Murgatroyd and Hawking (1931a) used the term Minimum Effective Dose to mean the minimum dose which sufficed to clear the blood of at least 80 per cent. of the treated animals, irrespective of time. In our case, time was a factor which had to be considered, as periodic remissions tended to occur with the less virulent strains. For this reason the sterilising dose was arbitrarily fixed as the minimum dose which would suffice to clear the blood of 80 per cent. of the treated animals within 3 days.

The results of these observations are shown in the accompanying Tables. It will be seen that there was a tremendous variation in the natural susceptibility to tryparsamide. Strains Gadau 4 and Ayu 5 were very sensitive to tryparsamide, being killed by 0·004 and 0·008 grammes per kilogram of body weight respectively, whereas Ayu 6 was comparatively resistant, the sterilising dose being in excess of 0·25 grammes per kilogram. The other Nigerian sleeping sickness strains occupied intermediate position between these two extremes. It was rather curious to find that, while the old *rhodesiense* strain was comparatively resistant, the old London *gambiense* strain was still fairly sensitive.

REACTION TO HUMAN SERUM *IN VITRO*

All the strains were tested against normal human serum according to the *in vitro* technique described by Yorke, Adams and Murgatroyd (1930). One part of diluted blood containing 20,000,000 trypanosomes

TABLE II

Showing the action of tryparsamide on strains Gadau

Strains	Date	Dose in grms. per kilo. of body weight	No. of guinea-pigs	Trypanosomes in peripheral blood. No. per field 1/6 obj. No. 5 oc.	No. which became neg.	Time in hours	Length of time guinea-pigs remained neg. (days)
Gadau 1	8/1/30	·04	5	.	2	24-48	1 : 16 1 : 6
Gadau 2	16/12/30	·04	5	. .	5	6-24	1 : 12 1 : 6 1 : 37 1 : 8 1 : died
Gadau 3	15/12/30	·04	5	. .	5	6	1 : 32 1 : 25 3 : died
Gadau 4	21/8/30	·04	4	.	4	6	1 : 31 1 : 30 1 : 37 1 : died
	2/9/30	·008	3	.	3	12-24	1 : 7 1 : 9 1 : 10
	25/9/30	·004	5		4	24-72	1 : 11 2 : 9 1 : 13
Gadau 5	3/12/30	·04	3	...	3	6	1 : 30 1 : 37 1 : died
Gadau 6	14/12/31	·04	3	.	0	...	...
	8/1/32	·08	5	.	5	6-24	1 : 11 1 : 17 1 : 9 2 : 10
Gadau 7	2/9/32	·04	3	5-20	1	72	1 : 10
	30/9/32	·04	5	3-20	3	24-48	1 : 21 1 : 13 1 : 18
	12/1/33	·06	5	...	4	...	1 : 3 1 : 4 1 : 7 1 : cured

Results : Sterilising dose : Gadau 1 >·04 grms. per kilogram.
„ 2 <·04 „ „
„ 3 <·04 „ „
„ 4 ·004 „ „
„ 5 <·04 „ „
, 6 ·04-·08 „ „
„ 7 ·06 „ „

TABLE III

Showing action of tryparsamide on strains

Strains	Date	Dose in grms. per kilo. of body weight	No. of guinea-pigs	Trypanosomes in peripheral blood. No. per field 1/6 obj. No. 5 oc.	No. which became neg.	Time in hours	Length of time guinea-pigs remained neg. (days)
Ayu 5	30/8/32	·04	5	4-20	5	6-9	1 : 28 1 : 27 1 : 7 1 : 79 1 : died
	20/9/32	·02	5	4-20	5	6-24	1 : 15 1 : 5 1 : 13 1 : 14 1 : died
	24/10/32	·15	5	1-20	5	24-48	1 : 7 1 : 23 1 : 22 1 : 29 1 : died
	29/12/32	·01	5	1-20	5	24-48	2 : 3 1 : 4 1 : 32 1 : died
	17/1/33	·005	5	10-20	1	24	1 : 3
	11/2/33	·008	5	11-20	4	24-72	2 : 3 1 : 4 1 : 16
Ayu 6	14/12/31	·04	3	...	0	...	...
	4/1/32	·25	5	...	1	24	1 : 12
Ayu 7	23/8/32	·04	5	2-20	2	6-12	1 : 35 1 : cured
	26/9/32	·04	5	7-20	1	48	1 : 4
	17/10/32	·06	5	4-20	5	24-72	1 : 5 1 : 7 1 : 3 2 : 4
Ayu 9	17/10/32	·04	5	1-10	2	24-48	1 : 5 1 : 11
	12/1/33	·06	5	1-20	5	12-48	3 : 7 1 : 3 1 : 2

Results : Sterilising dose : Ayu 5 ·008 grms. per kilogram.
„ 6 >·25 „ „
„ 7 ·06 „ „
„ 9 ·06 „ „

TABLE IV

Showing action of tryparsamide on strains

Strains	Date	Dose in grms. per kilo. of body weight	No. of guinea-pigs	Trypanosomes in peripheral blood. No. per field 1/6 obj. No. 5 oc.	No. which became neg.	Time in hours	Length of time guinea-pigs remained neg. (days)
Ayu 8	30/8/32	·04	5	5-20	0	...	...
	16/8/32	20	3	10-20	3	24	1 : 19 1 : 15 1 : 17
	5/9/32	·08	5	5-25	2	48	1 : 10 1 : 4
	20/9/32	·12	5	1-10	2	72	1 : 6 1 : died
	11/10/32	·16	5	5-20	5	24	1 : 22 1 : 11 1 : 23 1 : 26 1 : died
	14/11/32	·14	5	1-20	5	24	1 : 20 1 : 21 1 : 11 1 : 12 1 : 30
Ayu 11	26/9/32	·04	5	8-20	4	24-48	1 : 15 1 : 16 2 : 9
	11/10/32	·03	5	3-20	1	72	1 : 5
London *rhod.*	14/12/31	·04	4	0	0	...	...
	29/12/31	·25	5	0	0	...	...
London *gamb.*	16/12/31	·04	5	...	4	6-12	...
Brucei 3	12/11/32	·30	5	...	4	24	1 : 4 1 : 5
Brucei 4	9/11/32	·03	5	...	5	24	1 : 11 1 : 17 1 : 15 2 : 19
	28/12/32	·25	5	...	4	24-48	1 : 6 1 : 5 1 : 8 1 : 3

Results : Sterilising dose :	Ayu 8	·14	grms. per kilogram.
	„ 11	·04	„ „
	London *rhodesiense*	>·25	„ „
	„ *gambiense*	·04	„ „
	Brucei 3	·30	„ „
	„ 4	·25	„ „

per c.c. was mixed with 19 parts of fresh human serum and inoculated at 37° C. It was found to be essential that the serum should be quite fresh. Laveran and Mesnil (1902) state that human serum does not lose its trypanocidal power for some time unless it is contaminated by moulds or bacteria. Their observations were confirmed by Goebel (1907), Leboeuf (1911) and Adams (1928). Razgha (1929) refers to Mesnil's later work as showing that serum loses its power to damage trypanosome if it is kept for several days, and mentions that Zeiss and others were not able to confirm this. Here it was found that the trypanocidal power of human serum taken with aseptic precautions and kept on ice diminished on standing. Observations were repeated several times and the decrease of trypanocidal power was well marked in all cases, even though the sera appeared quite sterile. Details of such an experiment are given in Table V, which shows the effect of keeping human serum on ice for 6 days.

TABLE V

A comparison between the trypanocidal action of fresh human serum and that of the same serum kept on ice for 6 days

Date	Human serum		No. of living trypanosomes per 256 squares of the haemocytometer scale				
			Start	3 hrs.	6 hrs.	9 hrs.	24 hrs.
12/1/33	Adamu	taken 12/1/33	82	0	...	...	...
	Ozegiya		88	1	...	. .	...
	Ibrahim		90	0	...	...	...
	Sheep		86	84	89	73	33
18/1/33	Adamu	taken 12/1/33 and kept on ice for 6 days	66	16	9	4	0
	Ozegiya		60	29	15	13	0
	Ibrahim		69	31	13	5	0
	Sheep		50	61	57	60	32

Some European sera were used in these experiments, but for the main part the donors were African members of the Gadau staff. Taken as a whole there were no obvious differences in trypanocidal power between European and African sera.

It should be mentioned that at one period considerable difficulties were encountered. In the cool seasons of the year numerous tests had been carried out, both by the writer and by Dr. R. D. Mackenzie, and there had been no trouble in keeping trypanosomes alive in the control sheep sera. During April and May, 1932, when the daily maximum shade temperature varied between 100° and 110° F., results obtained by both workers were very irregular. It was often quite impossible to keep trypanosomes alive in sheep's serum for more than 6 hours. Heating the serum for half an hour at 64° C. seemed to make matters a good deal worse. Results with individual sheep were very variable and it was noticed that after a sheep had been bled a number of times its serum often showed a falling off in the power of supporting trypanosomes *in vitro*. For the time being, *in vitro* experiments had to be discontinued.

In July, after the onset of the rains, the weather was a great deal cooler, and from then onwards the writer had no further difficulty with the controls. A large number of sheep's sera were tested, and it was not found possible either by frequent bleeding or by heating the sera to effect the power of supporting trypanosomes. No explanation can be given for these phenomena, though it might be pointed out that Mackenzie and Marshall (1930) at Gadau found that guinea-pigs were deficient in complement during the hot season and that the titre increased as soon as the cold weather set in. It is now realised that it is difficult, if not impossible, to do serological work here at the hottest time of the year, and any results obtained during these two or three months have to be discarded. During the remainder of the year similar difficulties are not encountered.

The results of typical experiments done with each strain are given in Tables VI and VII. It will be seen that both Ayu 6 and Gadau 6 were very sensitive to normal human serum, practically all the trypanosomes being killed within 3 hours. The old London strains were sensitive to a similar degree, while the remainder of the human strains were completely insensitive. Of the two *brucei* strains, nos. 1 and 2 were killed off within 6 hours, while nos. 3 and 4 were killed in 3 to 4 hours.

TRYPANOCIDAL POWER OF INDIVIDUAL HUMAN SERA

An attempt was made to find out whether any considerable proportion of the local population showed a decrease or loss of serum trypanocidal power. Sera from 5 Europeans, 107 normal Africans

TABLE VI

Showing the action of normal human serum on strains *brucei* 1 to 4 and Gadau 1 to 5

Strain	Date	Serum	Number of living trypanosomes per 256 squares of the haemocytometer scale				
			Start	3 hrs.	6 hrs.	9 hrs.	24 hrs.
Brucei 1	6/8/30	H.M.O.L.	37	24	3	0	...
		Adamu	61	14	1	0	...
		Usman	76	16	2	0	...
		Melle	76	20	6	0	...
		Monkey control	116	107	106	98	...
Brucei 2	8/10/30	Dodo	53	15	9	0	...
		Isa	38	8	0	...	...
		Hama	33	11	3	0	...
		Monkey control	43	40	42	47	...
Brucei 3	3/11/32	Bulama	58	15	0	...	...
		Bako	43	11	0	...	...
		Musa	20	7	0	...	...
		Sheep control	32	30	28	26	6
Brucei 4	3/11/32	Bulama	50	4	0	...	...
		Bako	64	1	0	...	...
		Musa	39	0	0	...	...
		Sheep control	82	89	100	91	47
Gadau 1	5/11/30	Bako	57	52	49	45	22
		Usman	62	43	40	38	0
		Bekete	51	51	53	46	15
		Musa	51	44	46	36	0
Gadau 2	8/11/30	Bako	51	53	37	37	12
		Adamu	50	49	39	31	0
		Bekete	41	36	49	35	5
		Musa	55	43	33	32	...
Gadau 3	6/11/30	Bako	47	72	73	57	25
		Usman	66	68	72	64	0
		Bekete	64	71	68	73	25
		Musa	58	56	47	41	17
Gadau 4	24/10/30	Adamu	35	39	34	32	...
		H.M.O.L.	43	37	40	36	...
		Hardo	30	36	32	38	...
		Sheep control	46	43	41	47	...
Gadau 5	7/10/30	Bako	65	47	42	41	11
		Usman	51	50	46	29	0
		Bekete	56	56	60	53	15
		Musa	60	72	57	55	0

TABLE VII

Showing the action of normal human serum on strains Gadau 6 to 7, London *gambiense* and *rhodesiense* and Ayu 5, 6, 7, 8, 9 and 11.

Strain	Date	Serum	Number of living trypanosomes per 256 squares of the haemocytometer scale				
			Start	3 hrs.	6 hrs.	9 hrs.	24 hrs.
Gadau 6	21/12/31	Emanuel	61	4	0	...	...
		Paul	46	1	0	...	...
		Bulama	43	0	0	...	...
		Sheep control	46	49	41	...	...
Gadau 7	9/8/32	Adamu	46	34	29	30	18
		Bulangu	39	36	37	39	26
		Isa	25	49	34	29	20
		Sheep control	26	22	17	13	19
London *gambiense*	10/12/31	A.J.	64	0	...	...	...
		Adamu	47	0	...	...	...
		Bako	38	0	...	...	...
		Sheep control	33	35	39	...	...
London *rhod.*	15/12/31	H.M.O.L.	54	0	...	...	...
		Adamu	67	0	...	...	...
		Bako	55	3	...	...	...
		Sheep control	60	55	63	...	...
Ayu 5	5/8/32	Dodo	77	102	100	98	60
		Bulama	64	105	96	102	54
		Bako	75	89	80	96	52
		Sheep control	56	90	96	104	49
Ayu 6	10/12/31	A.J.	38	3	0	...	...
		Adamu	37	1	0	...	...
		Bako	43	0	0	...	...
		Sheep control	39	37	43	...	...
Ayu 7	13/8/32	Musa	62	79	83	73	25
		Abdala	67	81	82	75	29
		Garuba	89	88	110	87	27
		Sheep control	82	88	72	65	29
Ayu 8	5/8/32	Dodo	65	34	51	64	35
		Bulama	56	44	36	38	16
		Bako	68	67	60	65	22
		Sheep control	61	48	78	45	36
Ayu 9	9/8/32	Haruna	30	34	33	28	12
		Sale	38	43	37	32	10
		Bassey	32	30	20	13	11
		Sheep control	44	42	34	40	12
Ayu 11	5/8/32	Dodo	26	23	23	12	18
		Bulama	33	22	21	24	8
		Bako	26	17	20	15	6
		Sheep control	15	11	6	8	3

and 102 patients attending the Gadau dispensary were tested against strain *brucei* 4. The majority of the normal Africans were adult male labourers, while the patients were picked at random and included men, women and children. As far as possible, cases of sleeping sickness were excluded. Out of the total of 214 sera tested, only one, that from a case of secondary syphilis, showed any marked diminution in the trypanocidal power. With this serum trypanosomes survived for 9 hours, whereas with normal serum they were destroyed within 3 to 4 hours.

TABLE VIII

Showing the diseases from which the 102 patients were suffering

Disease	No. of cases tested	Disease	No. of cases tested	Disease	No. of cases tested
Syphilis	29	Dysentery ...	3	Abscess	1
Guinea worm ...	6	Gastritis	3	Elephantiasis ...	1
Tropical ulcer ...	7	Lymphosarcoma	1	Arthritis ...	1
Chronic bronchitis	5	Middle ear disease	2	Pyelitis	1
Sleeping sickness	4	Disseminated sclerosis ...	2	Epilepsy ...	2
Malaria	5	Constipation ...	1	Injury	1
Gonorrhœa ...	6	Variocele ...	1	Senility	2
Leprosy	3	Hernia	2	Cardiac disease	2
Liver abscess ...	1	Eczema	2	Tuberculosis ...	1
Catarrhal jaundice	1	Schistosomiasis ...	2	Splenic anaemia	1
Conjunctivitis ..	1	Ascites	1	Rheumatism ...	1

HUMAN INOCULATION EXPERIMENTS

Various workers, Taute (1913), Taute and Huber (1919), Curson (1928) and Duke (1932) have attempted to infect men experimentally with *T. brucei* by the injection of living trypanosomes. Up to the present, such experimental inoculations have yielded negative results. It was decided to carry out an experiment on similar lines at Gadau. Three Europeans and 40 natives were injected with blood from a white rat infected with *brucei* 4. Doses of blood containing approximately 10,000,000 living trypanosomes were inoculated into the arm of each volunteer. Immediately prior to inoculation the strain was tested against normal human serum which was found to destroy the trypanosomes within 3 to 4 hours. It should be mentioned that all the native volunteers were members of the Tsetse Investigation Staff and had been made quite familiar with the details of the proposed experiment. They were given monetary rewards which, to them, were adequate compensation for possible slight risk. As was anticipated, the results were negative in every case.

In making a strain of *T. brucei* resistant to human serum in mice, Lester (1932) noticed that a strain completely resistant in mice to a dose of 1 c.c. of normal human serum showed no signs of this resistance when tested *in vitro*. This finding was not reported at the time, but it has since been confirmed with another *brucei* strain. Strain *brucei* 4 was maintained in mice, which were treated with increasing intra-peritoneal injections of normal human serum, until such time as it had become completely resistant in the mouse to a dose of 2 c.c. of serum, which is about the maximum dose a mouse can tolerate. The resistant strain from a mouse and the normal strain from a guinea-pig were both tested against normal human serum, and little, if any, difference could be seen in their behaviour. The details of this test are given below.

TABLE IX

Showing the action of normal human serum *in vitro* on strain *brucei* 4 and on the same strain which had been made serum-resistant in mice

Strain	Date	Serum	No. of living trypanosomes per 256 squares of haemocytometer scale			
			Start	3 hrs.	6 hrs.	9 hrs.
Brucei 4 from guinea-pig	25/1/32	Dau	72	4	0	
		Umaru	67	3	0	...
		Adamu	96	5	0	...
		Sheep	88	92	101	96
Brucei 4 completely resistant in mice to a dose of 2 c.c. of normal human serum		Dau	84	17	0	...
		Umaru	60	0	0	...
		Adamu	89	0	0	..
		Sheep	58	52	67	63

TABLE X

A summary of the characteristics of the various strains

Strain	Virulence in small laboratory animals	Occurrence of posterior nuclear forms	Dose of tryparsamide required to clear trypanosome from the peripheral blood, grm. per kilogram	Reaction to human serum
Gadau 1	Low virulence	Normal	Greater than ·04 grms.	Insensitive
„ 2	Normal	„	Less than ·04 gms.	„
„ 3	„	Numerous up to 15 per cent.	„ ·04 „	„
„ 4	„	Normal	·004	„
„ 5	„	Up to 4·5 per cent.	Less than ·04 grms.	„
„ 6	Virulent	Numerous up to 23 per cent.	·08	Sensitive 3 hours
„ 7	Normal	Normal	·05	Insensitive
Ayu 5	„	Numerous up to 17 per cent. in white mice	·008	„
„ 6	Virulent	Numerous up to 29·4 per cent.	Greater than ·25 grms.	Sensitive 3 hours
„ 7	Normal	Normal	·06	Insensitive
„ 8	„	„	·14	„
„ 9	„	„	·06	„
„ 11	„	„	·05	„
London *gamb. T.*	Virulent	...	Less than ·04 grms.	Sensitive 3 hours
London *rhod. T.*	„	...	Greater than ·25 grms.	Sensitive 3 hours
Brucei 1	„	...	Greater than ·25 grms.	Sensitive 6 hours
„ 2	„	Numerous	·25	Sensitive 6 hours
„ 3	„	„	·30	Sensitive 3 to 4 hrs.
„ 4	„	„	·25	Sensitive 3 to 4 hrs.

This finding throws some light on Collier's experiment (1924), in which he failed to infect himself and 4 others with a strain of *T. brucei* which had been made serum-resistant in animals. His experiment was repeated here. A dose of 10,000,000 trypanosomes, resistant to human serum in mice, was injected into 1 European and 5 native volunteers. The results were again completely negative.

SUMMARY OF CHARACTERISTICS OF STRAINS

The accompanying Table gives a summary of the characteristics of the different strains. It will be seen that Gadau 6 and Ayu 6 appear to have the characteristics of *T. rhodesiense*, while Gadau 1, 2, 4 and 7 and Ayu 7, 9 and 11 have the characteristics of *T. gambiense*. The position of strains Gadau 3 and Ayu 5 and 8 is not quite so clear.

DISCUSSION

The original object of these observations was to find out whether *T. rhodesiense* occurs in Nigeria. Details of an exhaustive series of tests have been given, and it seems that not only do strains having all the characteristics ascribed to *T. rhodesiense* occur but that there is also a whole series of strains with characteristics intermediate between *T. rhodesiense* and *T. gambiense*. It is, perhaps, worth while re-opening the question of the relationship between *T. rhodesiense* *T. gambiense* and the game trypanosome, and discussing this vexed problem in the light of these findings.

CHARACTERS ASCRIBED TO *T. RHODESIENSE*

Trypanosoma rhodesiense was first isolated by Stephens and Fantham in 1910, from a sleeping sickness case in Rhodesia. They found that their trypanosome differed from *T. gambiense* in that it was much more virulent to small laboratory animals, and stained blood films from small animals contained numerous short forms with a characteristic posterior displacement of the nucleus. It was observed that *T. rhodesiense*, both in man and experimental animals, was resistant to treatment with arsenical compounds. The character of the disease in man seemed different from that caused by *T. gambiense*, *rhodesiense* sleeping sickness being much the more acute disease. It was believed to occur only in areas where game is numerous and to be carried only by the game tsetse *G. morsitans* and *G. swynnertoni*. Yorke and his co-workers, finding that their laboratory strains of *T. rhodesiense* were very sensitive to human serum *in vitro*, suggest that when this trypanosome is kept in small laboratory animals it soon loses its resistance to human serum and becomes serum-sensitive. In this way *T. rhodesiense* again differs from *T. gambiense*.

In practice, the most important character used to differentiate *T. rhodesiense* from *T. gambiense* seems to have been the occurrence of posterior nuclear forms. Archibald (1922) described the occurrence of a case of *rhodesiense* infection in the Sudan ; he based his diagnosis solely on the virulence and morphology of the causal trypanosomes in small laboratory animals. Archibald and Riding (1926) reported the discovery of a second case of this disease in the Sudan. On this occasion the diagnosis was based on the virulence and morphology of the trypanosomes, its resistance to atoxyl and the severity of the disease in the patient himself. Duke (1930) described the discovery of *T. rhodesiense* in man in Uganda ; he used virulence in laboratory animals, reaction to drugs, the occurrence of posterior nuclear forms and the adhesion test to differentiate his strain from *T. gambiense.*

It is interesting to analyse the characteristics which differentiate these two species of the human trypanosome. Apparently *T. gambiense* should have a low virulence to laboratory animals and posterior nuclear forms should either be absent or very scanty ; it should be sensitive to arsenical compounds, both in man and experimental animals. In man, the disease should be of a comparatively mild type, showing typical late nervous manifestations, the vector being *G. palpalis* or allied species. On the other hand, *T. rhodesiense* ought to be very virulent to small laboratory animals and to show numerous posterior nuclear forms. It should be comparatively resistant to arsenical compounds, and in man the disease should be of the acute type in which toxic symptoms predominate, patients dying before they have time to show typical nervous manifestations. In this case, the vector should be one of the game tsetse, *G. morsitans* or *G. swynnertoni.*

VIRULENCE

Dealing first with the question of virulence, it is well known that, just as with bacteria, the virulence of any particular trypanosome strain is far from being a constant factor, and it is often quite easy to alter virulence by one means or another.

Duke (1923) discussed the significance of virulence in the classification of the trypanosomes and concluded that it was quite unreliable. Thomson and Robertson (1926) showed that by mechanical transmission a strain of trypanosomes maintained in a series of hosts of the same species usually will exhibit increased virulence, though occasionally the virulence may actually decrease. Passage through a host of a species different from the usual may cause the virulence to be markedly modified, either diminished or increased. As has already been mentioned, Kligler and Rabinowitch (1927) have shown that the severity and speed of onset of an infection may be controlled by the size of the infecting dose. The greater the initial dose of trypanosomes the more acute will be the nature of the infection. They point out that the smaller the initial dose the greater is the possibility of the factors of inherent individual resistance coming into play.

Schilling (1930) found that a laboratory strain of *T. brucei* was far different in virulence from a freshly isolated strain, and that one cyclical passage of the old laboratory strain through Glossina sufficed to restore its original characteristics.

The writer (1932) found that a single passage through *G. tachinoides* profoundly modified the virulence of a strain of *T. brucei*, though the serum-resistant properties of this particular strain remained unaffected. Taylor and Mackenzie, working in the Gadau laboratories, carried out some interesting and as yet unpublished work with the old London strain of *T. rhodesiense*. Although this old strain had been kept in laboratory animals for many years, they were able to secure cyclical passage through *G. tachinoides*. Prior to this passage the strain was very virulent to both rats and guinea-pigs, but afterwards they found that its pathogenicity had changed and that it had become almost avirulent to both these animals. At first the writer was led to believe that cyclical transmission has such a marked balancing effect that any acquired virulence must be lost and the stability of the strain maintained. Later work has shown that this is not always the case. Two other strains of *T. brucei* have been passed through *G. tachinoides*, and although there was some falling off in virulence, this was not so well marked as to affect the whole course of the infection in mice, as happened in the first experiment. Similarly, with the human strain Ayu 5, which had been kept going for over a year in a series of guinea-pigs, and had so acquired an exalted virulence, cyclical transmission did not produce any well-marked change. It is noteworthy that in these latter experiments the method of securing cyclical transmissions described by Taylor (1932) was followed. In each case the tsetse were kept in an incubator at 37° C. for 6 days after the first infecting feed. In the early experiments the tsetse were kept at ordinary laboratory temperature, and it is probable that under such conditions cyclical transmission was produced by the bite of one or two infected flies only. Lloyd and Paisley (1929) concluded that the number of trypanosomes which are injected at a single bite of a tsetse carrying a swarming infection of *T. congolense* or *T. vivax* would not be greater than 1,000. It is likely that the number of trypanosomes injected by a fly infected with the polymorphic trypanosomes will be even less. In the later experiments, 200 tsetse were used for each transmission and the infection rates may have been anything up to 70 per cent. Under these conditions each guinea-pig would receive a very much greater infecting dose of trypanosomes. It seems possible that it is the size of the infecting dose which plays a big part in modifying the virulence of an infection produced by cyclical transmission, in much the same way as it does with mechanical transmission, although the possibility that there may be an actual change in the virulence of individual trypanosomes cannot be excluded.

The fact that virulence is a factor which can be altered, both by direct and cyclical transmission, indicates that difference in virulence between individual strains can hardly be of much value in demarcating species.

The Occurrence of Posterior Nuclear Forms

The occurrence or otherwise of posterior nuclear forms is by no means a specific character. Wenyon (1912) pointed out the insufficiency of posterior displacement of the nucleus as the distinctive characteristic of *T. rhodesiense.* Oehler (1914) and Reichenow (1921) considered that differences in morphology are to be correlated with differences in virulence. Duke (1923) agreed with this view, and came to the conclusion that it is unjustifiable and misleading to endow this character with any specific value. He wrote : ' I feel confident that epidemic strains of *T. gambiense* will be found in *palpalis* areas in the Congo, and that these, under suitable handling, will yield posterior nuclear forms.' Thomson and Robertson (1926) found that with the passage of time strains mechanically transmitted may change entirely in their morphological characters. They were able to show that changes in morphology can be brought about by the action of drugs.

Lavier (1927), working with guinea-pigs, demonstrated the presence of posterior nuclear forms in 7 strains of *T. gambiense* isolated from *G. palpalis* areas near Lake Victoria. He concluded that these forms normally exist in *T. gambiense* and that no value should be attached to them as a specific characteristic. Kleine (1927), working at a focus of *gambiense* sleeping sickness on the Kavirondo coast, also found posterior nuclear forms in his strains. In 1928, he paid a visit to the *rhodesiense* focus at Ikoma and examined a number of strains with the object of determining whether the percentage of nuclear displacement renders possible differential diagnosis between *T. rhodesiense* and *T. gambiense.* He found that not only were there great variations between strains but also that great differences could be noted at different times within an individual strain.

Similar results were obtained with our Nigerian strains, and it seems very difficult in the face of this evidence to assign any particular significance to nuclear displacement.

CHARACTERISTICS OF THE DISEASE IN MAN

It has long been recognised that the human disease in *rhodesiense* areas is of a more serious type than in *gambiense* areas. At the same time, the differences in the character of the disease are of doubtful significance when it comes to the question of differentiating one species of trypanosomes from another. Kleine (1928) compared the cases that he met with in the *rhodesiense* focus at Ikoma with those he had seen previously in *palpalis* areas. He pointed out that in the Sese Islands, on Lake Tanganyika and on the Belgian Congo, he had chiefly seen cases with marked glandular enlargement and with all degrees of involvement of the central nervous system. Patients in whom the disease progressed so rapidly that there was little time for clinical signs of central nervous system involvement to be apparent were scarce. At Ikoma, the latter type of the case was in the majority. In spite of these differences, he considered that one epidemic could not clinically be separated from another. None of the patients at Ikoma would have been considered as showing unusual symptoms if seen in the other area, but would only have been regarded as cases in which the disease was particularly severe.

In Nigeria, when only those cases coming in voluntarily to seek treatment could be examined, it could be said that the majority of cases showed severe glandular swellings and had nervous symptoms of all degrees up to profound mental disturbance. At the Tsetse Investigation headquarters at one time diagnosis was made almost entirely on the results of gland puncture, fresh blood films being examined only occasionally when there was reason to suspect an early case. Later, when thick blood film as well as gland juice examination became the routine method of diagnosis, many cases were found with little or no sign of glandular enlargement. When once the system of mass examination was inaugurated, every man, woman and child in a district being examined, the clinical picture was quite different. We can now divide cases roughly into three classes : (1) the type commonly met with in the field, in which the disease runs a very chronic course lasting several years ; patients may have few or no symptoms and present few clinical signs, though an examination of cerebro-spinal fluid commonly shows signs of involvement of the central nervous system ; (2) the type, commonly met with among patients seeking treatment voluntarily, in which the disease runs a fairly chronic course ; in the early stages the patient may have well-marked toxic symptoms, but various nervous manifestations supervene, and these increase in severity until the death of the patient ; (3) the type, much less common, in which the disease runs an acute course, patients dying within a few months of the onset. In some of these cases, severe nervous manifestations come on early, in others the patients die before showing obvious clinical signs of nervous involvement. These clinical types occur both in the epidemic areas in the northern part of the country and in endemic areas further south, where disease, taken as a whole, is much milder. In places where the disease has assumed the virulent epidemic form, class 2 is common and class 3 not very rare. Where the disease appears mild, class 2 is very much less common and class 3 is very rare, the majority of cases being of the very chronic type described as class 1.

It is interesting to compare the disease as seen in Nigeria with the *rhodesiense* sleeping sickness described by Buchanan (1929) as occurring in an epidemic focus in Tanganyika Territory. He divided his cases into three distinct clinical types : (1) that in which cases run a very acute course and if untreated die in from 2 to 3 months ; (2) that in which the disease takes a chronic form ; after an insidious onset, followed by a period of remission, the progress of the disease is very slow, the patients being able to carry on their usual occupations for many months ; (3) the type in which, after an acute onset, there are few signs of systemic disturbance but with marked symptoms and signs of central nervous system involvement. It is apparent that type 1 of the Nigerian cases correspond roughly with type 2 of Buchanan's cases, type 2 with his type 3, and type 3 with his type 1.

The real difference appears to lie in the fact that in Nigeria the milder types of the disease are the most common, whereas in the *rhodesiense* area the acute type of the disease is the most common.

RESISTANCE TO ARSENIC COMPOUNDS

Although at one time a natural resistance to arsenical compounds was regarded as one of the criteria of *T. rhodesiense*, for some years workers in the field have been meeting with cases of *gambiense* sleeping sickness which appear completely resistant to treatment with tryparsamide. Duke (1930), in reporting his discovery of *T. rhodesiense* in Uganda, mentions that he had isolated three strains from patients resistant to treatment with this drug. When these strains were investigated in the laboratory one of them was found to be *T. rhodesiense*, while the other two behaved like typical *T. gambiense* except for their abnormally high resistance to arsenical drugs. When we came to consider the Nigerian strains we found that there were great variations between the individual strains. Some were very sensitive to tryparsamide, others were moderately sensitive, and yet others had a comparatively high degree of natural resistance. In view of the above, resistance to arsenical compounds can hardly be regarded as a significant characteristic.

VECTOR OF THE TRYPANOSOME

T. rhodesiense is said to be carried invariably by one or other of the game tsetse. This again is rather a doubtful point. Duke (1930) records that he and Van Hof recovered, in 1929, a strain from a native woman in the Kilo Minawa, near the western border of Uganda, and that this strain proved indistinguishable from *T. rhodesiense*; *G. pallipides* and *G. palpalis* and one or more of the big forest species were the only tsetse known to occur in that part of the Congo. Among our strains, Ayu 6 has all the properties of *T. rhodesiense*, and yet it was isolated from a district where the vector is *G. palpalis*, the nearest known focus *G. morsitans* being many miles away. It might be argued that the particular strain had just undergone a laboratory transmission with *G. morsitans* and that it might have been this transmission through *G. morsitans* which produced the characteristics typical of *T. rhodesiense*. This possibility, although unlikely, cannot be excluded; but if this is actually what did happen, that is, if a single cyclical transmission could be sufficient to convert a strain of

T. gambiense into a strain of *T. rhodesiense*, obviously these two trypanosomes would have to be one and the same thing.

RELATIONSHIP BETWEEN *T. GAMBIENSE* AND *T. RHODESIENSE*

Soon after *T. rhodesiense* was described, Kinghorn and Yorke (1912) came to the conclusion that no differences exist between this trypanosome and *T. brucei*. Bruce and his co-workers (1913) reached the same conclusion. Other observers, Taute (1913), Kleine (1914) and Beck (1914), did not agree, but believed that the human trypanosome *T. rhodesiense* was a different species from the game trypanosome *T. brucei*. Ever since there has been a controversy as to the nature and origin of *T. rhodesiense*. At the present time there are three main schools of thought. Brumpt (1924) and the French workers believe that *T. gambiense*, *T. rhodesiense* and *T. brucei* are three distinct species and as such cannot be converted one into the other. Kleine (1928) considers that the two human trypanosomes are identical but are essentially different from the game trypanosome, *T. brucei*. Yorke and his co-workers (1929) have expanded the old theory of the British workers : they suggest that the human trypanosomes are only strains of *T. brucei* which, by one means and another, have acquired a resistance to human serum. *T. rhodesiense* is the form which has only recently come from game and so is still in the process of acquiring a resistance, while *T. gambiense*, being further removed from game, has a resistance which is much more of a fixed character. Duke (1928) has similar views : he believes that the differences that exist between *T. rhodesiense* and *T. gambiense* are only due to environment, and that *T. gambiense* and *T. brucei* are even more closely related to one another.

Now to consider the Nigerian strains. If Brumpt is correct and *T. gambiense* and *T. rhodesiense* are separate species, then Ayu 6 must be a strain of *T. rhodesiense*, somewhat abnormal as it occurred in *G. palpalis* country. Gadau 6 must be another strain of *T. rhodesiense*, atypical in that it had a comparatively low resistance to tryparsamide. Ayu 5, from *G. palpalis* country, and Gadau 3 and 6, from *G. tachinoides*, *G. morsitans* country, must be either abnormal strains of *T. gambiense* which showed numerous posterior nuclear forms, or atypical forms of *T. rhodesiense* of abnormally low virulence which has a lowered resistance to tryparsamide. Ayu 8 must be a strain of *T. gambiense* with an abnormally high resistance to tryparsamide.

Such a classification seems absurd: The various characteristics which have been used to differentiate *T. rhodesiense* from *T. gambiense* appear altogether unsatisfactory. The writer believes with Kleine that the two trypanosomes are identical. The trypanosome described as *T. rhodesiense* is nothing more nor less than a virulent strain of the ordinary human trypanosome *T. gambiense.*

RELATIONSHIP BETWEEN THE HUMAN AND GAME TRYPANOSOME

If this view is correct, there are still two ways by which the more virulent type (*T. rhodesiense*) may arise. Yorke and his school may be right, in which case the virulent strains are those which have been derived recently from game and so must be considered as strains of *T. brucei* which have only just gained a footing in man. The less virulent strains (*T. gambiense*) will be strains of the game trypanosome, whose characteristics have been altered to some extent by prolonged sojourn in man. On the other hand, if Kleine is right and the human trypanosome is altogether distinct from the game trypanosome, then the more virulent strains must be ordinary *T. gambiense* which have been modified by their environment, probably, as suggested by Buchanan (1929), by the factors particular to host resistance.

The main point against Yorke's theory is that so far no one has succeeded in infecting man experimentally with a strain of *T. brucei.* Professor Lefranchi was infected accidentally in Europe by a laboratory trypanosome strain, but there has always been some doubt as to the nature of the trypanosome concerned. Mesnil (1930) describes the case of one of Vauchel's laboratory assistants who became infected accidentally with an old laboratory strain of what was believed to be *T. brucei.* In the numerous inoculation experiments which have been carried out from time to time there has never been a single positive result. It has been argued—and quite rightly—that these failures mean very little ; probably the subjects of these experiments were persons whose sera had normal trypanocidal properties, in which case positive results could hardly be expected.

On the surface, Collier's findings, confirmed here at Gadau, seem to indicate that the natural trypanocidal power of the serum is not the factor which prevents the infection of man with *T. brucei.* The writer's observations that a strain of *T. brucei* resistant to a full dose of human serum in a mouse showed little sign of this resistance when tested *in vitro* against pure human serum, seems to throw light on this point. The strain resistant *in vivo* did not differ when tested *in vitro* from the normal strain, and so it is hardly to be wondered at that the inoculation of this apparently resistant strain did not give results differing from those of previous experiments. Further work will be required before it is possible to determine the cause of this discrepancy between the *in vitro* and *in vivo* findings. As has been stated by many workers, the mouse may play some part in the reaction. Alternatively, trypanosomes in a mouse from the nature of things can only be exposed to the action of very much diluted human serum, and the resistance which is acquired may be too small to show when tested against pure serum *in vitro*.

Corson (1932) succeeded in infecting himself with a strain of *T. rhodesiense* which had been maintained for 12 months in sheep. At the time of inoculation this strain was partially sensitive to normal human serum, being destroyed within 6 to 7 hours. Corson's own serum appeared to have normal trypanocidal properties. Duke (1932) argues that these findings show that there is an inherent fallacy in Yorke's theory that the infectivity of man is determined by the trypanocidal properties of his serum, and that other factors must be looked for. However, this need not necessarily be so, as Corson's result can be accounted for if it is assumed that not all the trypanosomes had lost their resistance to human serum. The strain was still partially resistant ; as far as could be detected, all trypanosomes were destroyed within 6 to 7 hours ; but it is quite feasible that a minute proportion of the trypanosomes were still completely resistant, and that, inoculated into man, these were sufficient to produce the disease. De Kruif (1921) and Wilson (1928) have shown with bacteria that there is a true discontinuous variation in virulence. In any one culture the bacteria are either virulent or avirulent and there are no intermediate forms, the net virulence of the culture depending on the proportion of virulent to avirulent forms. Yorke, Murgatroyd and Hawking (1931b), from their observations on the action *in vitro* of various arsenical compounds, conclude that all individual trypanosomes are not equally sensitive to the action of a drug. They believe that the development of drug resistance is fundamentally the result of a process of mutation or gradual change, although the possibility cannot be excluded that under certain conditions selection may play some part. The writer suggests that their experiments do not rule out an all-or-nothing change as must occur in bacteria. With minimum doses of a drug a few trypanosomes might be changed by each exposure, while, with maximum doses, more trypanosomes would be changed at each exposure and many of the sensitive trypanosomes killed off. A similar process might occur when a trypanosome is acquiring or losing resistance to human serum, so that with Corson's strain the individual trypanosomes may not have been equally sensitive to human serum.

At present, Yorke's hypothesis, attractive though it is, can only be supported by indirect evidence, as it has yet to be proved conclusively that a strain of *T. brucei* can be made to infect man. Until this has been done, further progress cannot be made along these lines, as indirect evidence alone will not suffice. Fresh human inoculation experiments will have been undertaken, though there now seems to be little point in injecting more healthy volunteers with normal strains of *T. brucei.* There appear to be two lines of approach to the problem : either the injection into man of a strain of *T. brucei* completely resistant to pure human serum, or the injection of normal human serum into patients suffering from liver diseases, etc., whose sera have been proved to be completely deficient in trypanocidal power. For obvious reasons the second of these methods would be difficult of accomplishment, but there do not seem to be any insuperable reasons why the first should not be tried. The *in vivo* technique has been shown to be of no value in making a *T. brucei* strain completely resistant to pure human serum. There should be little difficulty in adapting for this purpose the *in vitro* technique used by Yorke, Murgatroyd and Hawking (1931b) for making trypanosomes fast to drugs. Once this has been done there is little doubt that volunteers could be obtained who would agree to the inoculation.

The probability is that under certain conditions *T. brucei* can adapt itself to man, and that originally the human trypanosome must have been evolved in this way. However, such a happening is likely to be a rare accident and hardly accounts for the frequent occurrence of the *rhodesiense* type of trypanosome. Out of the 13 Nigerian strains investigated, 2 appeared to be of this type, while another 4 had intermediate characteristics. When the sera from the local inhabitants were examined, it was found that, out of 107 healthy people and 102 patients suffering from various diseases, there was not one which had lost completely its trypanocidal power. In only one man was the trypanocidal power markedly diminished, a case of secondary syphilis whose serum killed a strain of *T. brucei* within 9 hours, whereas normal human serum killed it within 3 to 4 hours. Admittedly these numbers are small, but it is hard to believe that there can exist locally sufficient abnormalities in the trypanocidal power of the sera of the general population to account for the findings with the strains investigated. Again, Ayu 6, a strain having all the characteristics of the *rhodesiense* type, was isolated from a patient living in a district where there is little game and no *G. morsitans*. It is difficult to believe that the characteristics of this strain can be accounted for by its having come recently from game. It is easier to surmise that all these strains originated from the normal human trypanosome, and that the differences in their characteristics were due to idiosyncrasies in the resistance of the human host.

It will readily be admitted that the resistance of the host can produce marked changes in the characteristics of a trypanosome. There is a good deal of evidence to show that under certain conditions the human trypanosome can change into something very like *T. brucei*. Duke (1912 and 1921), from his studies of the Damba Island strains, has produced evidence that *T. gambiense* maintained solely in game and fly, and so lacking the stimulus of sojourn in the human host, has reverted back to *T. brucei*. It is well known that, where strains of *T. gambiense* are kept in laboratory animals for some years, they may lose their resistance to human serum. Mesnil and Blanchard (1916) mention one strain isolated by Mesnil which, after 7 years, had acquired a slight degree of sensitiveness to human serum, and after 11 years had become almost as sensitive as *T. brucei*. Razgha (1929) found that, while it was quite easy to culture a freshly isolated strain of *T. gambiense* in a medium containing human serum, it was very difficult to cultivate a strain which had been kept 8 years in the laboratory. He suggested that failure with the old strains was due to a gradual development of a susceptibility to human serum. As has already been mentioned, the old laboratory strain of *T. gambiense*, which had been kept in London in laboratory animals for 10 years, was found to be more sensitive to human serum than comparatively freshly isolated *T. brucei*.

It seems possible that when the human trypanosome gets into man, whose resistance for one cause or another is abnormal, the disease may take a virulent form. During the course of the disease the trypanosome may acquire an increased virulence, and its other characteristics may be altered in a manner comparable to that which is known to occur slowly when strains are kept for prolonged periods in small laboratory animals. The writer holds the view that *T. rhodesiense* is only a virulent type of *T. gambiense*, and that this more virulent type usually arises from the normal human trypanosome through idiosyncrasies in the resistance of the human host. At the same time, it is thought that under certain circumstances *T. brucei* may get a footing into man in accordance with Yorke's hypothesis, but that this occurrence may be comparatively rare.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS *MUSCA*, BASED ON A COMPARATIVE STUDY OF THE MALE TERMINALIA

II. A PRACTICAL GUIDE TO THE PALAEARCTIC SPECIES

(*Continued from page* 345)

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DOMESTICA GROUP

This group contains the simplest species, and there are three in this faunistic area, *domestica*, *vicina* and a species which for the present I am naming *nebulo* ; these three are closely related to each other.

1. **Musca domestica** L. Fauna Suec. Ed. II, 439, 1761.

SYNONYMS : *_antiquissima_ Wlk. ; ? _aurifacies_ R-D. ; *_australis_ Macq. ; ? _bovina_ R-D. ; *_calleva_ Wlk. ; ? _campestris_ R-D. ; ? _campicola_ R-D. ; ? _corvina_ F. ; ? _frontalis_ Rond. ; ? _floralis_ R-D. ; ? _hottentota_ R-D. ; ? _lateralis_ Macq. ; _ludifica_ F. ; *_minor_ Macq. ; ? _riparia_ R-D. ; ? _rivulans_ R-D. ; *_pampasiana_ Big. ; ? _pellucens_ Meig. ; ? _stomoxidea_ R-D. ; _umbraculata_ F. ; ? _vagatoria_ R-D. ; ? _vicina_ R-D. nec Macq. ; *_vicaria_ Wlk.

MALE. HEAD. Vertex approximately half width of an eye ; vertical stripe occupying almost whole of vertex ; cheeks and lower part of vertex creamy white. MESONOTUM. Ground colour dark grey with bluish tinge and four black stripes as wide as vertex.

ABDOMEN. Terga 1 and 2 dark brown to black and about posterior half orange on each side of broad black median stripe ; tergum 3 orange with broad black median stripe, both ends of which, especially the anterior, spread out along the margins, edged on each side with indistinct silvery stripes, and lateral margins of tergum with silvery dusting ; tergum 4 greyish, or greyish-white, a broad median black stripe edged on each side with a distinct wide white stripe, the lateral margins with a wide silvery stripe, and between the two a well-marked dark brown stripe ; tergum 5 similar but with indistinct median dark stripe, the admedian ones narrower. Sternum 1 dark brown ; sternum 2 also dark brown except sides and posterior border ; sterna 3 and 4 yellow ; sternum 5 with sides of distal end and posterior processes dark.

MALE TERMINALIA. P. I, fig. 5, *c*. ANAL CERCI. Free borders straight, outer ends rounded and inner not forming nipples. PHALLOSOME. Chitinous part stout and slightly longer than membraneous part ; posterior process stout, rather short, not expanded and pointed. PARAMERE. Anterior part large and well arched with one long and five to six smaller bristles ; posterior part small, round and not raised. FIFTH STERNUM. P. I, fig. 6. Wider than long and narrowing posteriorly ; posterior processes fairly long and minutely, but well, serrated.

FEMALE. HEAD. Vertex about equal to width of an eye ; vertical stripe wide almost filling vertex with bulging sides ; outer vertical bristles for the most part in two rows (fig. 2) ; lower part of vertex and cheeks creamy white. MESONOTUM. As in male. ABDOMEN. Terga 1 and 2 dark orange or dark brown, and often as in male, normally with a broad dark brown median stripe which expands as in male ; tergum 3 grey with bluish sheen in fresh specimens, narrow to broad median stripe, and narrow to broad dark admedian ones as in terga 4 and 5 of male.

EARLY STAGES. Oviparous, eggs laid in batches in horse and human faeces, rarely, if ever, in cow dung ; for list of other materials in which eggs may be laid see Part II of my book, ' Insects, Ticks, Mites, etc.' THIRD LARVA. Anterior spiracles either with six or seven finger-like openings ; in about 78 per cent. there are six and in about 28 per cent. seven, and it should be remembered that the number may not be the same on the two sides. POSTERIOR SPIRACULAR PLATES. Fig. 9, *c*. D-shaped, the flat surfaces directed towards each other and the plates usually separated by less than half the width of one. Peritreme wide ; button large and placed well within it ; the three breathing slits wide, sinuous and lying around the inner surface of the peritreme.

NOTES. *M. domestica* is the house fly of temperate regions, and as already noted (1931) is widely distributed in Europe, North and South America, Asiatic Russia, across to North India, China, Japan, Korea, and is common in Australia and New Zealand ; it is also widely distributed in South Africa. It is the only species known to me from the Nearctic and Neotropical regions. It is regularly carried in ships from European to tropical ports, and I have seen it carried as far as Colombo and Singapore. In this way it has been, and is, regularly introduced into such Indian ports as Bombay, Madras and Calcutta, and also into many African ports. I have no proof, however, that it has established itself at any of these and spread inland. After many years of collecting at Indian ports as well as inland, I have not been able to satisfy myself that it is anywhere established in India. For a complete modern account of the life history, early stages, relation to disease and control of *domestica* and its near allies, which are house- and bazaar-frequenting insects, the reader is referred to Part II of my book, ' Insects, Ticks, Mites, etc.'

2. ***Musca vicina** Macq., nec Rob-Des. Dipt. Exot. Nov. ou. poi. conn. Supp. V, 253, 1851.

SYNONYMS : *analis* Macq. ; *divaricata* Awati ; **flavifacies* Big. ; **flavinervis* Thom. ; **flavipennis* Big.

MALE. HEAD. Vertex approximately one-third width of an eye, and about one-third that of the male *domestica* ; vertical stripe occupying whole of vertex. MESONOTUM. Similar to that of male *domestica* but ground colour generally greyish-yellow. ABDOMEN. Similar to that of male *domestica* but more extensively orange ; terga 1 and 2 about half orange with a broad median black stripe, the remainder black ; tergum 3 more orange than in *domestica* ; sternum 1 dark brown ; sterna 2, 3, 4 and 5 light orange.

MALE TERMINALIA. Fig. 7. I have now examined about 50 examples of the terminalia of *vicina* from different parts of the tropics and especially from India, and have compared them with specimens of those of *domestica*. In fig. 7 are illustrated typical examples of the phallosome, paramere, anal cerci and fifth sternum of *vicina* from South India. These illustrations should be compared with those in P. I, figs. 5, 6, which depict typical examples of the same structures of *domestica* drawn and reduced to the same scale ; the following differences may be noted :—ANAL CERCI. Smaller outer ends of free margins more rounded and the margins not so straight. PHALLOSOME. Chitinous part narrower ; posterior process shorter and more broadly expanded. PARAMERE. Identical. FIFTH STERNUM. Smaller and narrower, otherwise similar.

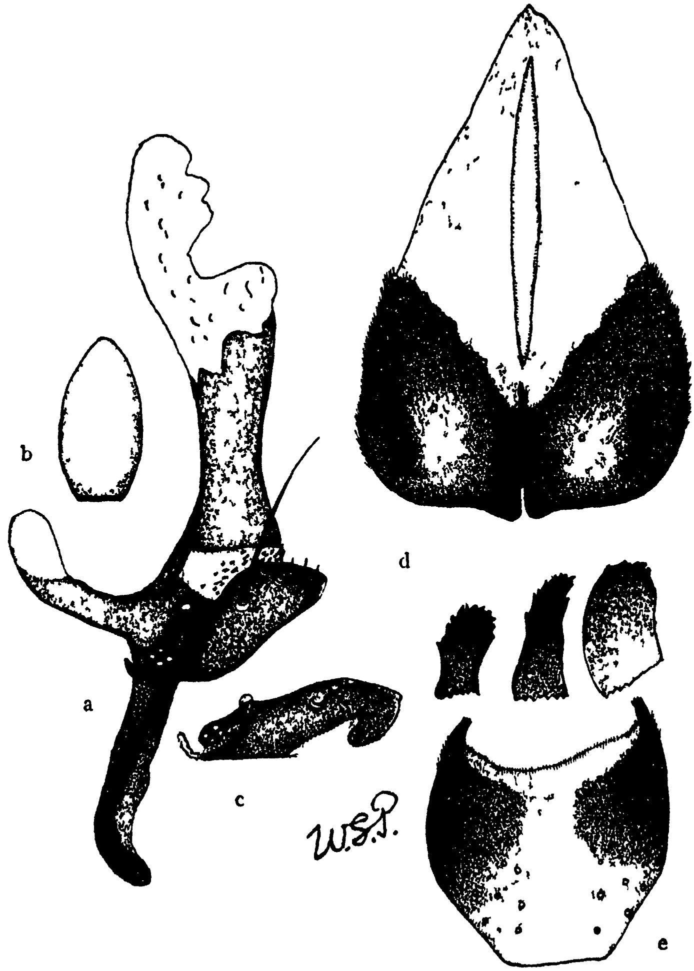

Fig 7 *a*—Phallosome and one paramere of *M vicina*, showing the short, stout posterior process, *b*—Front view of posterior process, *c*—Paramere of same from side, *d*—Anal cerci of same, *e*—Fifth sternum of same and enlarged posterior process of same, to show variation in structure

FEMALE. Very similar to that of *domestica*, so that it is almost impossible to distinguish the two. Terga 1, 2 and, especially, 3 are, if anything, more orange.

EARLY STAGES. Oviparous. Eggs laid in much the same substances as are those of *domestica*; they are commonly laid in large collections of cow dung. THIRD LARVA. Anterior spiracles with five to seven finger-like openings. POSTERIOR SPIRACULAR PLATES Fig. 9, *a*. General structure similar to those of larva of *domestica* but larger and peritreme wider.

NOTES. *Musca vicina* is the common house-fly of the tropics. It comes into the region in the Mediterranean area and is common in Egypt, Palestine and Iraq ; also in North China.

3. **Musca nebulo** Fabricius. Ent. Syst. IV. 321, 35, 1794.

MALE. HEAD. Vertex narrow, about one-fifth width of an eye ; lower part of vertex and cheeks creamy. MESONOTUM. Ground colour grey with four black stripes about twice as wide as vertex. ABDOMEN. Mainly orange ; terga 1 and 2 with broad median black stripe, and about half anterior border black ; tergum 3 light orange with a broad median black stripe edged with a rather indefinite, and sometimes incomplete, white stripe on each side, silvery marginal patches ; tergum 4 very similar, but with much more silvery markings and with the median black stripe narrower ; tergum 5 entirely

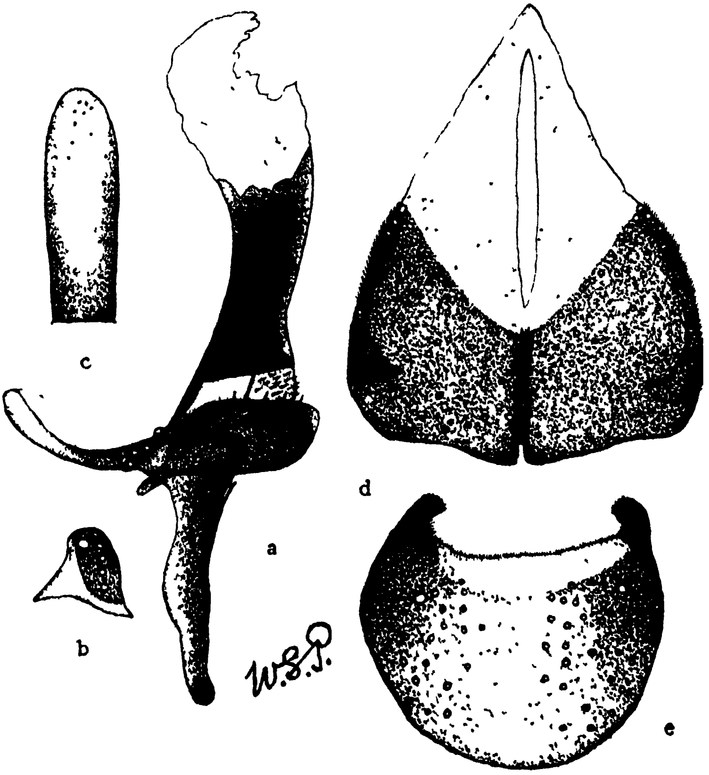

FIG. *a.*—Phallosome and one paramere of *M. nebulo* (Egypt) ; *b.*—Front view of anterior part of paramere of same, to show bristles ; *c.*—Front view of posterior process of phallosome of same ; *d.*—Anal cerci of same ; *e.*—Fifth sternum of same.

silvery, or sometimes with a median black area not forming a very definite or complete stripe.

MALE TERMINALIA. Fig. 8. ANAL CERCI. Free margins with shallow emargination, outer ends rounded and inner not forming nipples. PHALLOSOME. Chitinous part long and with slight waist ; posterior process rather short, stout and not expanded. PARAMERE. Anterior part long and deep with four to five, rarely six, bristles, one medium sized and the others small ; posterior part small and not raised. FIFTH STERNUM. Short, broad and posterior processes stout and bent inwards.

FEMALE. HEAD. Vertex and cheeks creamy white, less than width of an eye ; vertical stripe wide with slightly bulging sides, and about three-quarters width of vertex. MESONOTUM. Similar to that of male. ABDOMEN. Light orange ; terga 1 and 2 orange with a small round or oval median dark brown spot on anterior border (the size of the spot varies considerably) ; tergum 3 with narrow median dark stripe, which may be almost wanting, bordered on each side by a broad silvery or greyish-white stripe, margins white or greyish-white ; tergum 4 very [illegible]

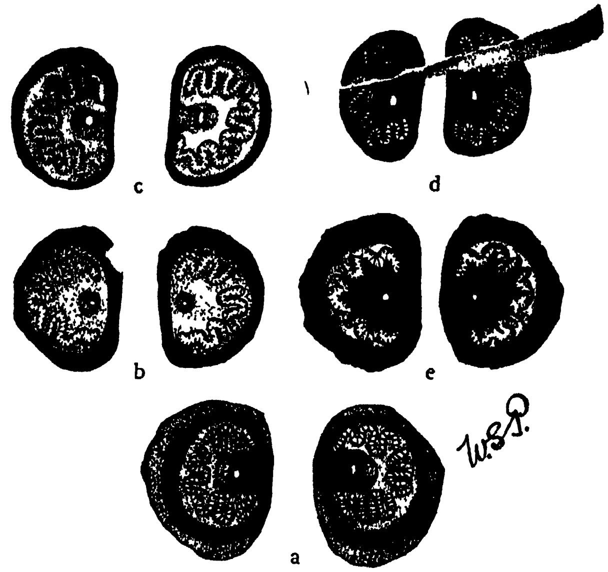

FIG. 9. *a.*—Posterior spiracles of third larva of *M. vicina* ; *b.*—Same of third larva of *M. ventrosa*; *c.*—Same of third larva of *M. domestica*; *d.*—Same of third larva of *M. tempestiva* ; *e.*—Same of third larva of *M. sorbens* (all same scale).

stripe often very faint, remainder of tergum light orange or greyish-white ; tergum 5 mainly orange.

Early Stages. Unknown.

Notes. Although this species differs in certain details (external characters and terminalia) from the true *nebulo* from India, I have decided not to make it a new species until I have had an opportunity of examining a fresh series and of comparing more specimens of the terminalia than I have had the opportunity of doing as yet. It appears to be widely distributed in Egypt and the Sudan.

SORBENS GROUP

This group contains the intermediate species in which the posterior part of the paramere exhibits the early phases in its enlargement into a long process, and the posterior process of the phallosome is expanded hood-like, and in some species shows evidences of Y-forking. In a few species the inner end of the free margin of the anal cercus projects somewhat, suggesting the commencing formation of a nipple-like process. There are seven species of this group in this region, viz., *sorbens*, *ventrosa*, *vitripennis*, *albina*, *tempestiva*, *crassirostris* and *lucidula*.

4. ***Musca sorbens** Wied. Auser. Zwei. Insekt. II, 218, 58, 1830.

Synonyms : **angustifrons* Wlk. ; **biseta* Hough ; **bivittata* Thom. ; *conducens* Patton nec. Walk. ; **dichotoma* Bezzi ; **euteniata* Big. ; **humilis* Wied. ; **latifrons* Wied. ; **mediana* Wied. ; *promusca* Awati ; *scapularis* Rond. ; **sordidissima* Wlk. ; **spectanda* Wied.

Male. Head. Vertex narrow, one-sixth to one-seventh or one-eighth width of an eye ; vertical stripe almost entirely filling vertex with straight edges ; lower vertex and cheeks silvery ; well-marked large facetted area on each side of inner eye margins. Mesonotum. Ground colour light grey with two broad black stripes not interrupted at suture. Abdomen. Mainly light to dark orange ; terga 1 and 2 either light or dark orange, and may even be entirely dark brown, antero-median border dark brown, sometimes with a faint indication of a narrow median dark stripe, or even only dark on antero-median border ; tergum 3 light orange, a broad black median stripe spreading out a little T-shaped along anterior border, edged on each side with a broad silvery stripe and with silvery marginal patches ; tergum 4 with a narrower dark median stripe and silvery patches as in tergum 3; tergum 5 with a median dark patch, sometimes forming a narrow dark stripe, and margins of tergum silvery.

MALE TERMINALIA. P. I, figs. 12, 13. ANAL CERCI. Free borders straight, outer ends rounded and inner ones not forming nipples. PHALLASOME. Chitinous part narrow and shorter than membraneous portion; posterior process long, rather narrow and slightly expanded at end, with a suggestion of commencing forking. PARAMERE. Anterior part rather long and not much arched, with one long and four to seven smaller bristles; posterior part raised to form a cone-shaped process; bluntly pointed and of varying length, and occasionally flattened at the end. A reference to the legend of P. I, fig. 12, will show that I have compared the paramere of specimens from Sierra Leone, where the type specimen came from, with that of specimens from India and Mesopotamia, and also with that of specimens with light and dark terga 1 and 2, and can find no differences in them. FIFTH STERNUM. Long, dark brown at distal end, processes long, serrated.

FEMALE. HEAD. Vertex a little less than width of an eye; vertical stripe rather narrow and about half width of vertex; outer vertical bristles in a single row, except at upper end where there is usually another irregularly placed row. MESONOTUM. Ground colour similar to that of male with two broad dark stripes which, anterior to the suture, divide Y-shaped, the outer limb of the Y being shorter than the inner, and not extending more than half the length of the inner stripe. ABDOMEN. Mainly dark orange, the posterior part yellowish; terga 1 and 2 either entirely dark brown, or anterior and posterior borders dark brown and middle portion orange; tergum 3 with a broad median dark brown stripe commonly extending along both anterior and posterior borders to form narrow dark brown stripes of varying width but usually wider posteriorly, and a wide marginal yellowish-white stripe on each side; tergum 4 very similar, the median dark stripe narrower, the admedian spots and stripes varying in width according to the angle at which the specimen is held; tergum 5 with a narrow dark median stripe and dark admedian spots.

EARLY STAGES. Oviparous, eggs laid in batches in either horse, human or cow faeces, and commonly in isolated patches of these in the field. It is not uncommon to find many thousands of larvae in small deposits of human faeces. THIRD LARVA. Anterior spiracles with seven to eight finger-like openings. POSTERIOR SPIRACULAR PLATES. Fig. 9, *e*. Relatively large, separated by about one-quarter width of a plate, peritreme wide and with irregular outline ; breathing slits wide.

NOTES. *Musca sorbens* is widely distributed along the North African coast, in Egypt, Palestine, Syria, Iraq, Arabia, Persia, across Asiatic Russia to North China. Owing to its extensive breeding habits, it is a very common domestic species and readily enters houses. It is, however, most commonly seen on foodstuffs in bazaars, and sitting on sores, cuts, wounds, etc., of man and animals. In Iraq it undoubtedly plays an important part as a mechanical transmitter of the causal organisms of eye diseases. It is quite common to see a dozen or more sitting around the eyes of a child, feeding on the discharges. It can become a most annoying pest about bungalows if cattle are allowed to graze in the neighbourhood, for it has the objectionable habit of alighting on the human skin in search of moisture. Dwarfed specimens are very common, owing to the fact that large numbers of eggs are commonly deposited in isolated patches of human faeces, and, as the large number of larvae are unable to obtain sufficient food to reach full growth, they are forced to pupate prematurely, and then give rise to small adults ; these are most annoying, as they persist in searching for moisture on the human skin. Adults may be seen far from human dwellings.

The general greyish colour, the two broad, complete mesonotal stripes in the male, and the Y-forked presutural stripe in the female render identification easy. The width of the male vertex varies considerably (fig. 1, *a*, *b*), as does also the colour of terga 1 and 2.

5. ***Musca ventrosa** Wied. Ausser. Zwei. Insekt. II, 656, 17, 1830.

SYNONYMS : *kasauliensis* Awati ; **nigrithorax* Stein ; *pungoana* Karsch ; **xathomela* Walk.

MALE. HEAD. Vertex linear (fig. 1, *c*) ; lower vertex and cheeks silvery ; large facetted area. MESONOTUM. Ground colour dark slate blue, with four broad black stripes commonly appearing to be fused. ABDOMEN. Orange, and without any stripes or bands, sometimes with a faint indication of a median stripe on terga 3 and 4 (dark intestinal contents must not be mistaken for markings).

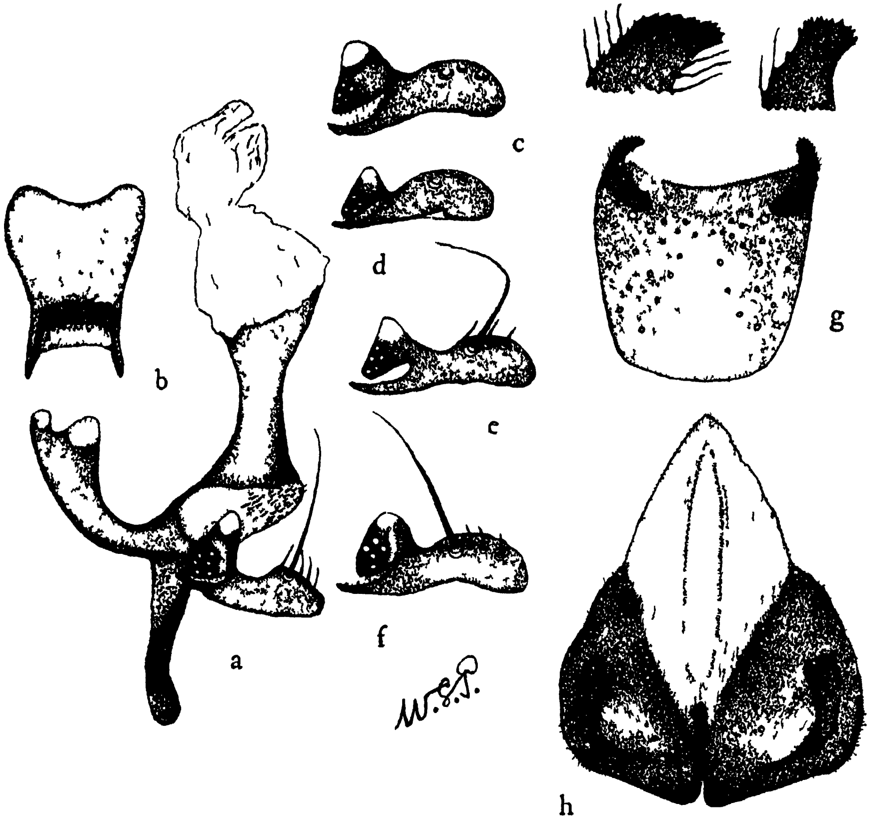

FIG 10 *a*—Phallosome and one paramere of *M ventrosa*, *b*—Front view of posterior process of phallosome, note expansion and slight forking, *c*—Side view of paramere of same (Africa), *d*—Same of specimen from China, *e*—Same of specimen from India, *f*—Same of specimen from Iraq, *g*—Fifth sternum of same with processes shown enlarged, *h*—Anal cerci of same

MALE TERMINALIA Fig 10 ANAL CERCI Similar to the other species of the group, the outer ends rounded and the inner not forming nipples PHALLASOME Chitinous part narrow and with distinct waist, and about as long as, or if anything a little shorter than, the membraneous part, posterior process rather short, well expanded at end and with suggestion of forking PARAMERE Anterior part of the usual shape with one long and three to four shorter bristles; posterior portion raised into a conical process of varying height, the apical part often clear FIFTH STERNUM Slightly longer than broad, the posterior processes rather short, but broad and serrated at ends

FEMALE HEAD Vertex wide, a little more than half the width of an eye; vertical stripe reddish, wide and about three-quarters width of vertex, sides slightly bulging ; outer vertical bristles mainly in a single row. MESONOTUM. Similar to that of male. ABDOMEN. Also similar to that of male.

EARLY STAGES. Oviparous. Eggs laid for the most part in patches of cow dung in the fields. THIRD LARVA. Anterior spiracles with five to seven finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 9, *b*. Smaller than those of *sorbens*, and separated by a little less than one-third width of a plate, peritreme wide.

NOTES. *Musca ventrosa* comes into the Palaearctic region in North China. It is haematophagous in habit and is only seen on and about animals in the field feeding on wounds, cuts, sores and on the blood exuding from the bites of true blood-sucking flies. It is one of the easiest species to recognise by its orange-coloured unmarked abdomen.

6. **Musca vitripennis** Meig. Syst. Besch. Europ. Zweiflug. Insekt. V. 73, 48, 1826.

SYNONYMS : **osiris* Wied. ; *phasiaeformis* Macq., nec. Meig. ; *sugalatrix* R-D

MALE. HEAD. Vertex narrow, about one-eighth, or even less, width of an eye ; eyes with numerous long hairs ; vertical stripe narrow, light grey. MESONOTUM. Dark metallic green without any markings ; two presutural dorsocentrals, usually long, and two postsuturals, and only one acrostichal. ABDOMEN. Mainly light orange or yellow ; terga 1 and 2 orange with a broad dark metallic green area occupying the middle and part of the anterior border ; tergum 3 with a broad dark green median stripe expanding anteriorly T-shaped along anterior border ; tergum 4 with a large silvery patch occupying almost whole of tergum except margins which are orange, a small dark green spot at middle of posterior border, and another at middle of anterior, sometimes the two are fused to form a narrow dark median stripe ; tergum 5 almost entirely silvery with metallic sheen, and sometimes with dark spots at posterior margin.

MALE TERMINALIA. P. I, fig. 23. ANAL CERCI. The free margins almost straight, outer ends not markedly rounded ; inner ends not forming nipples. PHALLOSOME. Chitinous part long and narrow with slight waist ; posterior process stout, and well expanded hood-like without suggestion of forking. PARAMERE. Anterior portion long, with one long and two to four shorter bristles ; posterior part raised and forming a long bluntly-pointed process often lightly chitinised. FIFTH STERNUM. Small and short, the posterior processes rather long and well serrated.

FEMALE. HEAD. Vertex grey, and about, or a little more than, width of an eye ; vertical stripe a little more than half width of vertex with bulging sides ; outer vertical hairs small and arranged in three and often four rows ; eyes bare. MESONOTUM. Greyish-blue with some metallic sheen and four distinct dark stripes ; dorsocentrals and acrostichals as in male. ABDOMEN. Light greyish-yellow or grey ; terga 1 and 2 grey with broad dark median patch or stripe, and with silvery sheen on each side, anterior border also dark ; tergum 3 grey with a narrow dark median stripe and some silvery sheen on each side ; tergum 4 grey and in some lights silvery, with a very narrow dark median stripe ; tergum 5 silvery and without any markings.

EARLY STAGES. Unknown.

NOTES. *Musca vitripennis* is widely distributed in Europe, especially in the south, in the whole of the Mediterranean littoral, Syria, Palestine, Iraq, Arabia, Persia and along the Indian frontier, Kashmir (Baniar, Kukarnag), Egypt and the Sudan. It is mainly found on and about animals in the field and about their excreta. It will readily enter trains. It is an annoying fly, as it settles on the human skin in search of moisture.

The male can easily be recognised by its hairy eyes, dark metallic thorax and yellowish abdomen with the characteristic silvery patches; the only other species like it is the male of *albina*, which, however, has greatly reduced mesonotal bristles, and the sternopleurals are wanting. The female may be mistaken for a large specimen of *tempestiva* which it resembles superficially. The wide vertex, rather narrow vertical stripe and the four rows of small outer vertical hairs will help to distinguish it from *tempestiva*, in which the vertex is narrower, the vertical stripe wider and the outer vertical bristles in a single row.

7. ***Musca albina** Wied. Auser. Zwei. Insekt. II, 415, 53, 1830.

SYNONYMS : *beckeri* Schnabl ; *polita* Mall. ; **sepculifera* Bezzi.

MALE. HEAD. Vertex narrow, about one-seventh or less width of an eye ; eyes with long hairs, not so numerous as in *vitripennis* ; cheeks silvery. MESONOTUM. Dark bluish-black, with metallic sheen and no markings ; a characteristic light grey patch under each posterior pronotum (humeral callus); mesonotal bristles much reduced, only one postsutural dorsocentral, and acrostichals wanting; sternopleurals wanting. ABDOMEN. Orange; terga 1 and 2 either entirely orange or with a dark median spot at middle of posterior border spreading slightly on to tergum 3; tergum 3 orange with a small round dark spot at middle of posterior border; tergum 4 orange with a large silvery median patch and a dark metallic spot at middle of posterior border; tergum 5 with a large silvery patch and two dark metallic admedian spots.

MALE TERMINALIA. P. I, fig. 16. ANAL CERCI. Outer margins of free borders rounded and inner not particularly prominent. PHALLOSOME. Chitinous part relatively long and about equal in length to the membraneous part; posterior process stout and somewhat broadly expanded hood-like with suggestion of forking. PARAMERE. Anterior part rather short, with one stout (and usually long) and four to five smaller bristles arranged usually in a line or nearly so; posterior portion raised to form a broad bluntly-rounded sugar-loaf process. FIFTH STERNUM. Rather small and short, the posterior processes long and slightly curved, and not markedly serrated.

FEMALE. HEAD. Vertex silvery, very wide, equal to width of an eye; vertical stripe strikingly narrow, a little more than one-quarter width of vertex; outer vertical hairs small and arranged in four or five rows; eyes bare. MESONOTUM. Greyish-white (silvery) with four broad dark shining stripes; mesonotal and sternopleural bristles as in male. ABDOMEN. Greyish- or yellowish-white with black markings; terga 1 and 2 black, commonly with a small anterior stripe of orange; tergum 3 greyish-white with a broad median dark stripe, a wide posterior black band, and a narrower anterior one; tergum 4 yellowish-white, either with a very narrow, complete, median dark stripe, or with a dark spot on postero-median border, and dark spot on each side of posterior border; tergum 5 yellowish-white with two small dark admedian spots.

EARLY STAGES. Unknown.

NOTES. *Musca albina* is very widely distributed in the Palaearctic, Ethiopian and Oriental regions. It is a semi-desert species. I have records of it from Colomon and Agouagon, Dahomey, where it is relatively common, from many localities in South and South-West Africa; from Mahagany and Trincomali, Ceylon; from Baluchistan.

It is common in Syria, Palestine, all along the North African coast, and in Egypt at such places as El Mallah and Kerdasa (Effletoun).

It is one of the most strikingly coloured species, the male rather dumpy, with dark metallic thorax and yellow abdomen, and the female silvery—characters which render their recognition easy. The male was not known to Wiedemann, and as far as I know the first description of it was by Patton and Senior-White (1924); the hairy eyes of the male were not noted in the description.

8. **Musca tempestiva** Fallen. Musc. 53, 56, 1823.

SYNONYMS: *carnifex* R-D.; *cuprea* Macq.; *minima* Rond.; *nana* Meig.; *stimulans* R-D.

MALE. HEAD. Vertex linear; lower part and cheeks silvery. MESONOTUM. Dark grey to black, with some metallic sheen; a light grey patch on posterior pronotum; vein M_{1+2} with rather a rounded bend. ABDOMEN. Mainly olive grey; terga 1 and 2 black; tergum 3 with a broad median black stripe, usually spreading out at both ends to form short dark bands; tergum 4 grey, usually with narrow to very narrow black median stripe, often incomplete at posterior end; tergum 5 grey, often with a dark patch about middle.

MALE TERMINALIA. Fig. 11. ANAL CERCI. General shape similar to those of other species of group; outer ends more or less

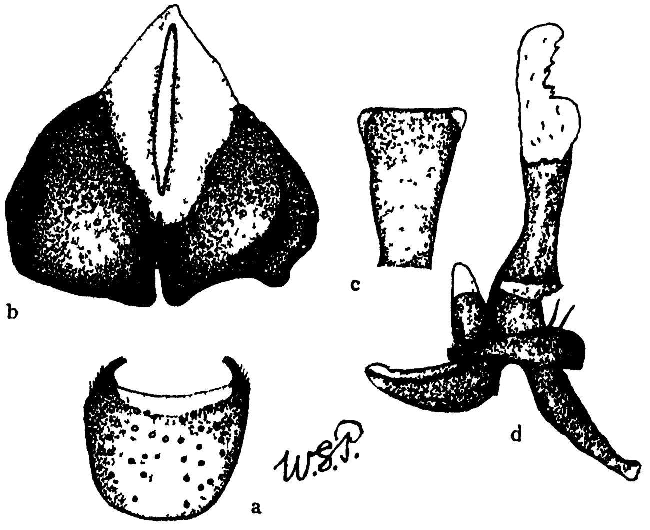

FIG. 11. *a.*—Fifth sternum of *M. tempestiva*, *b.*—Anal cerci of same; note asymmetrical left cercus; *c.*—Front view of posterior process of phallosome of same; *d.*—Phallosome and one paramere of same (Europe); note long sugar-loaf posterior part of paramere.

rounded, left cercus often with a suggestion of nipple formation, and not uncommonly asymmetrical, as illustrated ; inner ends not forming nipples. PHALLOSOME. Chitinous portion long and narrow, slightly longer than membraneous part ; posterior process short and wide, gradually expanding to end, without suggestion of forking. PARAMERE. Anterior part long with two, sometimes three, bristles, usually of same length and not very long ; posterior portion forming a long stout sugar-loaf process, the distal end clear. FIFTH STERNUM. Short and wide, posterior processes rather long and serrated at ends.

FEMALE. HEAD. Vertex wide, a little less than width of an eye ; vertical stripe broad, black, more than half, and sometimes nearly three-quarters, width of vertex with bulging sides ; outer vertical bristles in a single row. MESONOTUM. Yellowish-grey with four rather narrow stripes, the inner pair well marked in front of suture, but tending to join outer pair behind suture to form a single stripe. ABDOMEN. Very similar to that of male, except that black bands on tergum 3 are wider ; tergum 5 occasionally has a narrow median black stripe.

EARLY STAGES. Oviparous, eggs laid in batches in cow dung, especially in patches in the field ; also in horse and human faeces. THIRD LARVA. Anterior spiracles five to seven finger-like openings. POSTERIOR SPIRACULAR PLATES. Fig. 9, *d*. Small and rather long, and separated by about one-third width of a plate.

NOTES. *Musca tempestiva* is very widely distributed in Europe, extending down through Syria to Palestine, into Egypt, into Iraq, Arabia, Persia and all along the North-West Frontier of India into Kashmir, then on into China, where it is very common all over North and Central China. It is found on and about animals in the field, and on their excreta ; it is essentially a haematophagous species, and is a very annoying fly to human beings, settling on the skin in search of moisture. The characters by which the female may be distinguished from the female *vitripennis* have been noted above. There should be no difficulty in recognising the male, for it is not likely to be confused with the male of any other species in this region. The male terminalia are also very characteristic.

9. ***Musca crassirostris** Stein. Mitt. Zool. Mus. Ber. II, 99 139, 1903.

SYNONYMS : **insignis* (*Philaematomyia*) Aust. ; *modesta* de Meij. ; **inconstans* Wied. (nomen nudum).

MALE. HEAD. Vertex very narrow, about one-eighth or less width of an eye ; lower part of vertex creamy white ; cheeks greyish-white ; palps orange ; mentum bulbous, labella commonly drawn back and prestomal teeth everted (see fig. 5, this paper, and figs. 3, *a* ; 4, *a*, *b*, *e* ; 5*b*, 1933). MESONOTUM. Ground colour light grey (sometimes with a bluish tinge), and with four rather narrow black stripes ; outer pair interrupted at suture, presutural part short and directed outwards, inner pair rather narrow and hardly interrupted at suture, and commonly incomplete behind, just in front of scutellum. ABDOMEN. Olive or greyish-green ; terga 1 and 2 black except for a narrow greyish-green band along posterior border which may be interrupted in the middle line ; tergum 3 greyish-green, with a narrow median black stripe incomplete at posterior end ; tergum 5 greyish-green, often with either an antero-median dark spot or an incomplete, very narrow, dark stripe.

MALE TERMINALIA. P. I, figs. 18, 19. ANAL CERCI. Small, free borders rounded, especially outer. PHALLOSOME. Chitinous part long and narrow ; posterior process long, stout, expanded hood-like, rounded and no suggestion of forking. PARAMERE. Anterior part long and usually well arched, with one long and four to six shorter bristles, the variation in number and arrangement well illustrated in figs. noted ; posterior portion with a rather long finger-like extension which is very characteristic of this species and that of *lucidula* (see P. I, fig. 20) ; apodeme rather short and narrow. FIFTH STERNUM. Small, rather heart-shaped, the processes rather long and serrated.

FEMALE. HEAD. Vertex grey, about equal to width of an eye ; vertical stripe a little more than one-third width of vertex with straight sides ; outer vertical bristles in two rows ; antennae, palps and proboscis as in male. MESONOTUM. Ground colour olive grey to greyish-blue ; stripes much as in male, except that the inner pair are narrow in front of suture and are not as a rule interrupted behind suture as in male. ABDOMEN. Olive grey to greyish-blue with markings much as in male ; terga 1 and 2 with about anterior half black ; the remainder olive grey to greyish-blue ; terga 3, 4 and 5 as in male, except that there is rarely any median stripe or spot on tergum 5.

EARLY STAGES. Oviparous, egg usually large and hatches rapidly ; laid in masses, many females ovipositing together, in patches of cow dung in the field. THIRD LARVA. When mature is of a characteristic lemon-yellow colour. Anterior spiracles seven to eight finger-like openings. POSTERIOR SPIRACULAR PLATES. Fig. 12. Rather long and densely chitinised without definite peritreme, separated by about half width of a plate. The plates vary in size in larvae from different localities, those from Salisbury, Rhodesia (fig. 12, *b*), being as a rule very much larger than those from larvae from India (fig. 12, *a*).

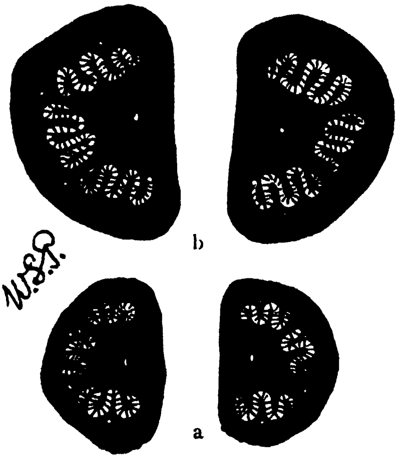

FIG. 12. *a*.—Posterior spiracles of third larva of *M. crassirostris* (India) ; *b*.—Same of third larva from Rhodesia ; note in both the absence of distinct peritreme.

NOTES. *Musca crassirostris* is widely distributed in Egypt, Palestine, Iraq, Persia and Arabia ; I collected it around Tsinan, Shantung, North China. Its tearing and blood-drawing apparatus has been recently described (1933). It is very closely related to *lucidula*. It is one of the commonest blood-sucking flies in the Ethiopian and Oriental regions and may be seen in large numbers sucking blood from the legs and abdomen of cattle, horses and donkeys. There should never be any difficulty in identifying it, for, apart from its characteristic colouration, the bulbous mentum is a striking character, even when the proboscis is completely retracted ; the usual appearance is depicted in fig. 5.

10. **Musca lucidula** Loew. Neu. Beit, IV, 48, 42, 1856.

Male. Head. Vertex silvery white, narrow, about one-eighth or less width of an eye; vertical stripe a dark line; well-marked large facetted area. Mesonotum. Bright metallic green and unmarked; bristles much reduced in size; vein M_{1+2} joining vein R_{4+5} and cell R_5 closed. Abdomen. Orange with metallic and silvery markings. Terga 1 and 2 light orange with or without a small triangular-shaped dark median patch; tergum 3 light orange with or without a narrow dark median stripe widening slightly anteriorly; tergum 4 orange with a green metallic patch at posterior border and silvery sheen at sides; when viewed with head directed away from observer, the patch then appears to be a median dark stripe edged with silvery sheen; tergum 5 green with a large light patch.

Male Terminalia. P. I, fig. 20. Anal Cerci. Small, outer ends of free margins rounded and inner ones not forming nipples. Phallosome. Chitinous part narrow with waist, and as long as membraneous part; posterior process, stout, well-expanded, hood-like and with no suggestion of forking. Paramere. Anterior part long and well arched, with one long and usually four to five shorter bristles; posterior part with a long finger-like extension very similar to that of *crassirostris*. Fifth Sternum. Small and heart-shaped, posterior processes rather long and serrated.

Female. Head. Vertex silvery, about as wide as, or a little less than, width of an eye; vertical stripe rather narrow and about one-third width of vertex, straight edges; outer vertical bristles in a single row. Mesonotum. Light metallic green with four rather indistinct narrow dark stripes. Abdomen. Bright metallic green; tergum 5 with a large silvery patch.

Early Stages. Unknown.

Notes. *Musca lucidula* is widely distributed in Europe, Syria, Palestine and Egypt. It is common on and about animals and is haematophagous in habit.

LUSORIA GROUP

This group contains the most advanced species of the genus in which the posterior part of the paramere is developed into a long, commonly bent, process, and the posterior process of the phallosome is widely expanded and, commonly, deeply Y-forked. The free end of the cercus is usually emarginated so that the inner end is produced into a nipple-shaped process, and the outer end may also be similarly shaped. There are six species in this region as follows :—*larvipara*, *convexifrons*, *mesopotamiensis*, *xanthomelas*, *hervei* and *autumnalis*.

11. **Musca larvipara** Portschinsky. Bur. Ent. Comm. Sc. Minist. Agr. St. Petersb., 8, No. 8, 13, footnote, 1910.

SYNONYM : *corvinoides* in litt., Schnabl and Dziedzicki (♂).

MALE. HEAD. Vertex silvery, linear, slightly wider than that of *autumnalis* but in all other respects very similar (see *autumnalis*) ; cheeks silvery. MESONOTUM. Ground colour bluish-grey with four broad black stripes. ABDOMEN. Orange (lighter than that of *autumnalis*) with white or creamy white markings ; terga 1 and 2 entirely black, or black except for a small dark orange area on postero-external border ; in some specimens this area is well marked, and in others it is so dark that it is difficult to make out its exact extent ; tergum 3 orange with a broad median dark stripe spreading out anteriorly to form a dark band, a silvery patch or stripe of varying extent on each side of median dark stripe, margins with silvery patches ; tergum 4 orange with a broad median dark stripe edged on each side with a wide silvery or creamy stripe, and silvery patches at margins ; tergum 5 with a dark patch in middle, lateral whitish or yellowish stripes and marginal white patches. Sterna 1 and 2 and distal end of fifth sternum dark brown.

MALE TERMINALIA. Fig. 13. ANAL CERCI. Large, free margins slightly emarginated towards inner ends, outer ends broadly rounded, inner ends forming short nipples. PHALLASOME. Chitinous part long and broad, and a little shorter than membraneous part ; posterior process long, broadly expanded, and well Y-forked. PARAMERE. Anterior part deep, well arched, projecting well backwards, most commonly with three long bristles (one longer than others), rarely four ; posterior portion long, bent, wide at base and narrowing towards apex, with four to six rather prominent sensory spines near base. FIFTH STERNUM. Relatively small, the processes very short, broad and bent inwards, as shown in illustration (cf. with those of *autumnalis*, fig. 22, *a*).

FEMALE. HEAD. Vertex creamy, about width of an eye ; vertical stripe about half width of vertex, and with slightly bulging sides ; outer vertical bristles in two rows. MESONOTUM. Greyish with four black stripes. ABDOMEN. Black with greyish-white markings ;

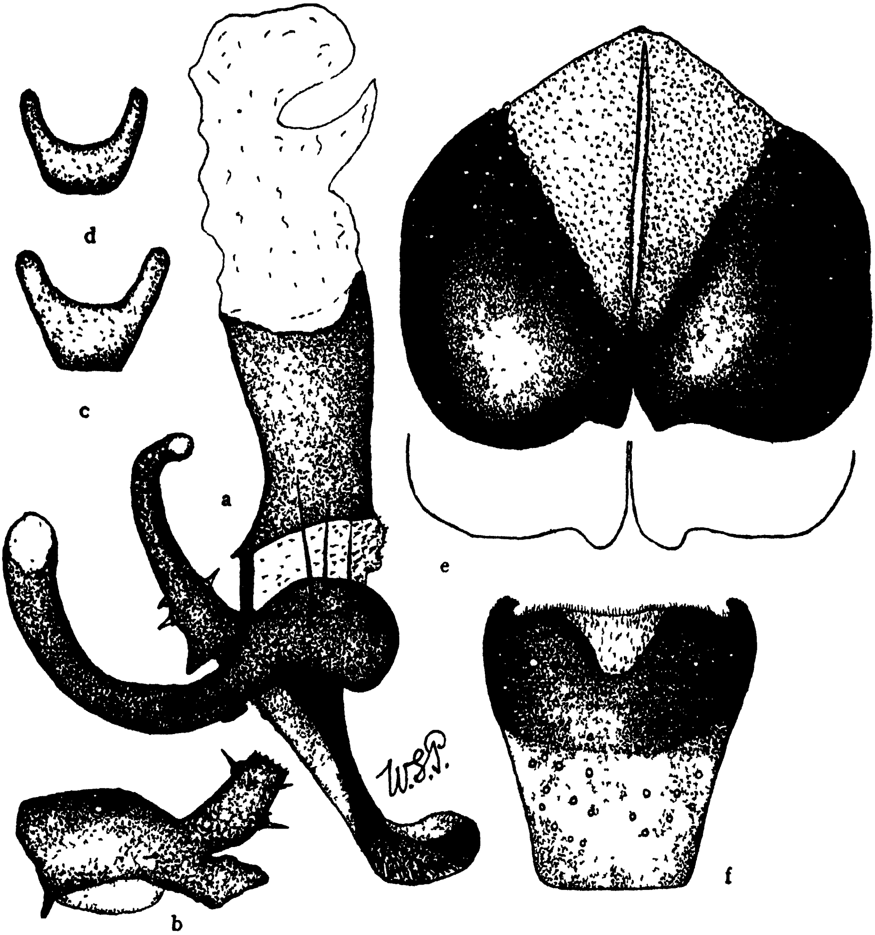

FIG. 13. *a.*—Phallosome and one paramere of *M. larvipara* ; note the long, rather narrow posterior part of paramere ; *b.*—Part of one paramere, to show spines at base of posterior part ; *c.*—Posterior view of posterior process of phallosome, to show expansion and Y-forking ; *d.*—Front view of same ; *e.*—Anal cerci of same and outline of free margins of another of same ; *f.*—Fifth sternum of same ; note short bent processes.

terga 1 and 2 black with a white or bluish-white patch on posterior border on each side of middle line and of varying size, sometimes quite long ; tergum 3 with a broad median black stripe edged with broad grey or white stripes, broad triangular-shaped admedian dark stripes and wide marginal white patches ; tergum 4 very similar, but median black stripe much narrower ; tergum 5 with a very broad median black stripe, and lateral silvery or grey patches ; all sterna black.

EARLY STAGES. In his studies of the species of *Musca* in the

Crimea, Portschinsky noted that *autumnalis* (his *corvina ovipara*) laid eggs in the usual way in the early spring and occasionally in the summer, but that towards the end of spring and throughout the summer larvae were almost exclusively deposited, and that these were in the third stage. In 1913 the late Professor Bezzi pointed out to me that it was most probable that Portschinsky was dealing with two distinct species, and this suggestion has been proved to be correct by Schnabl and Dziedzicki (1911). The puparium of *larvipara* is dark brown, whereas that of *autumnalis* is white.

NOTES. As noted above, it is extremely difficult to distinguish the male *larvipara* from the male *autumnalis* on external characters. The abdomen of *larvipara* is not of such a bright reddish colour as that of *autumnalis* ; the structure of the anal cerci and fifth sternum, however, afford easy characters for separating them, even in fresh specimens. The female is exactly like that of *larvipara*, being if anything larger.

12. ***Musca convexifrons** Thomson. Eugenies Resa. Dipt. 547, 176, 1868 (♂).

SYNONYM : *fergusoni* Patton nec. Johnston and Bancroft.

MALE. HEAD. Vertex linear, silvery ; cheeks silvery ; large facetted area present. MESONOTUM. Ground colour grey with four broad black stripes ; wing with a row of bristles on under surface of of vein R_{4+5}. ABDOMEN. Dark orange or brown with silvery markings; terga 1 and 2 dark orange with a black median stripe expanding a little posteriorly to form a short band, anterior half of tergum dark brown ; tergum 3 with a broad black median stripe edged with silvery stripes, wide, admedian, broad brown areas, margins silvery ; tergum 4 very similar but silvery markings more extensive, and posterior border edged with a narrow black band ; tergum 5 also similar but median stripe slightly narrower.

MALE TERMINALIA. Figs. 14, 15. ANAL CERCI. Very large, free margins also emarginated close to inner ends as in *larvipara*, outer ends similarly broadly rounded, inner ends forming prominent broad nipples. PHALLOSOME. Chitinous part long and broad, and shorter than membraneous portion ; posterior process broad, markedly bent forwards, expanded, and deeply Y-forked. PARAMERE. Anterior part long, deep, with three, rarely four, bristles, one much longer than others ; posterior portion not markedly elongated, wide and

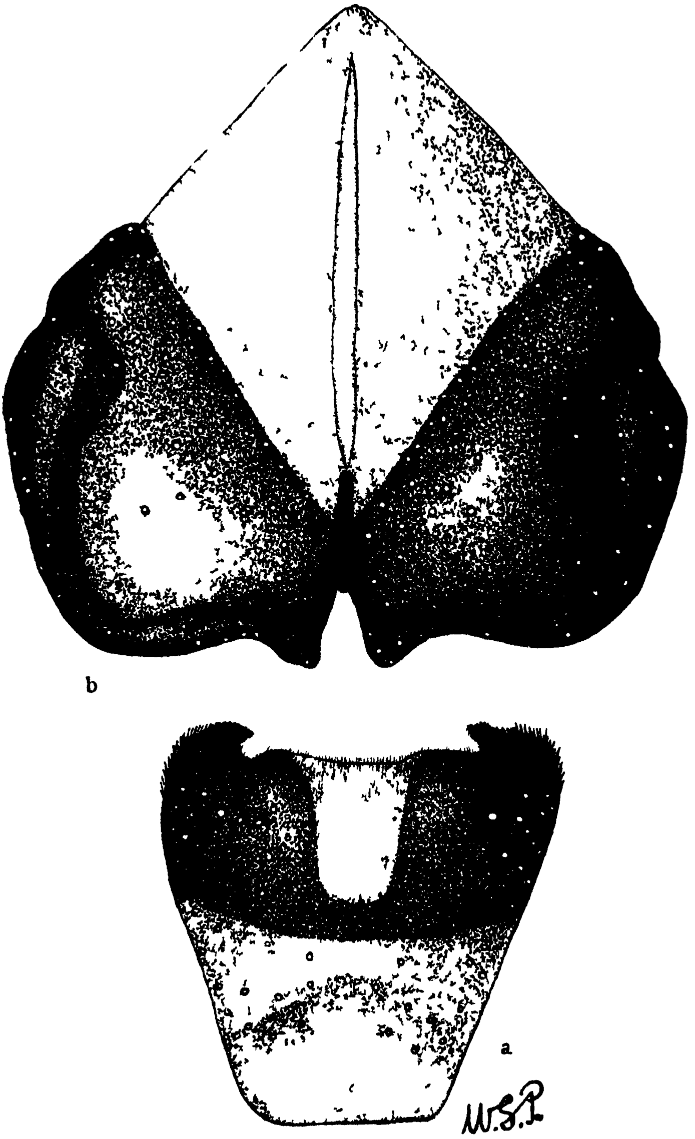

FIG 14 *a.*—Fifth sternum of *M. convexifrons* , compare with that of *larvipara* (same scale), and note large size and similar structure , *b* —Anal cerci of same , compare with those of *larvipara* (same scale)

well bent, with one, sometimes two, humps, and four to six sensory spines. FIFTH STERNUM. Large, long distal half dark brown as in *larvipara*, and posterior processes short, broad and bent inwards as in those of *larvipara*

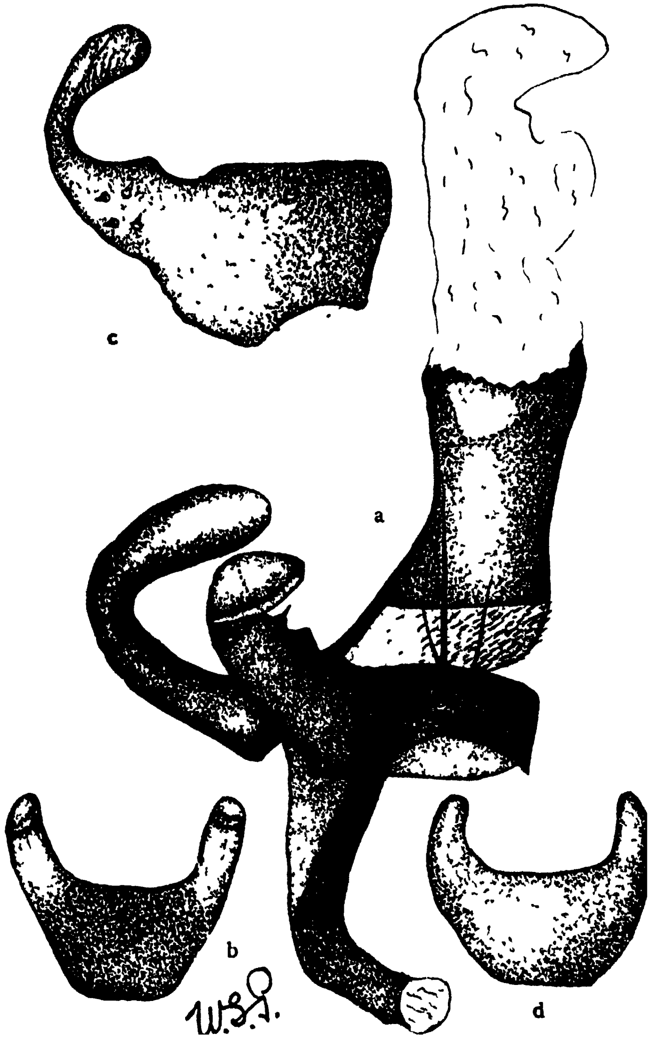

FIG. 15. *a* —Phallosome and one paramere of *M. convexifrons* , compare with those of *larvipara* ; *b*.—Front view of end of posterior process of phallosome of same ; *c*.—Side view of paramere of same ; *d*.—Posterior view of end of posterior process of phallosome of same.

FEMALE. HEAD. Vertex silvery, a little less than width of an eye ; vertical stripe rather narrow, about half width of vertex ; outer vertical bristles in two rows, and most long. MESONOTUM. Coloured and marked as in male. ABDOMEN. Black with grey markings ; terga 1 and 2 black with a large bluish-grey round spot or patch at posterior border on each side of middle line ; tergum 3 with a broad black median stripe, edged with wide silvery stripes, admedian dark stripes expanding posteriorly and margins silvery; tergum 4 very similar; tergum 5 also similar, but all stripes much narrower.

EARLY STAGES. Larviparous, larva deposited at commencement of second stage. Third stage larva and puparium not known.

NOTES. This large haematophagous species is very common in Shantung, North China. It may be seen in large numbers on cattle and other animals in the field, and on and about cow dung. In 1922, I examined Thomson's type and came to the conclusion that it was identical with the large haematophagous Australian species *fergusoni*; but, on examining the male terminalia of the two, I now see that they are distinct. There is no note with the type as to what part of China it came from. The female is very like the female of *larvipara*.

13. ***Musca mesopotamiensis*** Patton. Ind. Jl. Med. Res., VII, 4, 751, 1920.

MALE. HEAD. Vertex grey, about one-eighth width of an eye; cheeks grey. MESONOTUM. Ground colour bluish-grey with four black stripes. ABDOMEN. Light orange with extensive silvery markings; terga 1 and 2 light orange with a rather narrow median black stripe and anterior dark band; tergum 3 light orange with a broad median black stripe, spreading out along anterior border on each side of middle line T-like, edged with a broad white stripe and silvery patch at each margin; tergum 4 similar, but median dark stripe very narrow, posterior border of tergum with a narrow dark band, silvery markings extensive; tergum 5 similar, with an indistinct dark median stripe and silvery sides. Sterna 1 and 2 and distal end of 5 dark brown.

MALE TERMINALIA. Figs. 16, 17. ANAL CERCI. Outer ends of free borders broadly rounded, and inner ends with short nipples. PHALLOSOME. Chitinous part rather long and broad, but shorter than membraneous part; posterior process long, broadly expanded and deeply Y-forked; apodeme short. PARAMERE. Anterior part long and not very deep, with three bristles, one usually very long; posterior portion broad, almost same width throughout, and markedly bent. FIFTH STERNUM. About as wide as long, a little less than distal half dark, posterior processes short, broad and bent inwards.

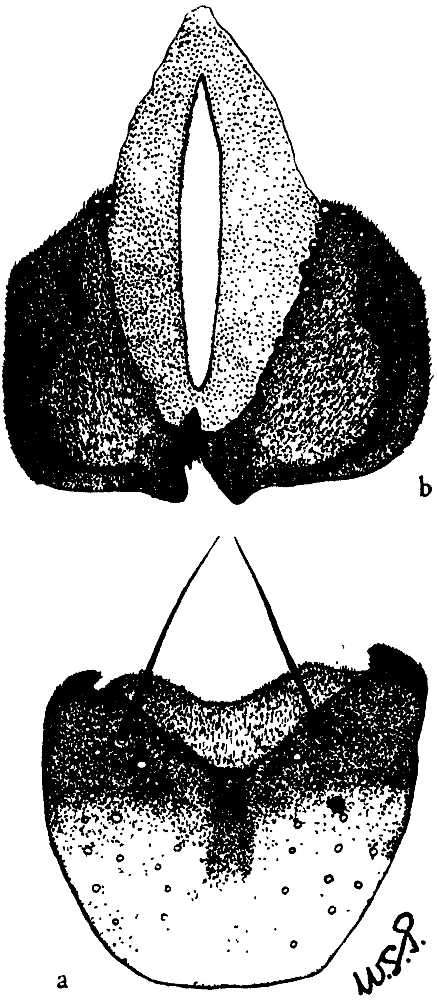

FIG. 16. *a*.—Fifth sternum of *M. mesopotamiensis* ; *b*.—Anal cerci of same.

FEMALE HEAD. Vertex grey, less than width of an eye ; vertical stripe black, rather narrow, about one-third width of vertex with straight edges ; outer vertical bristles in a single row. MESONOTUM. As in male. ABDOMEN. Black with grey markings ; terga 1 and 2 black with a large bluish-grey spot on each side of middle line, sometimes extending along posterior border ; tergum 3 with a narrow median black stripe edged with a broad bluish-grey stripe on each side, a dark admedian stripe, and bluish-white marginal patches ; tergum 4 very similar, but median black stripe much narrower ;

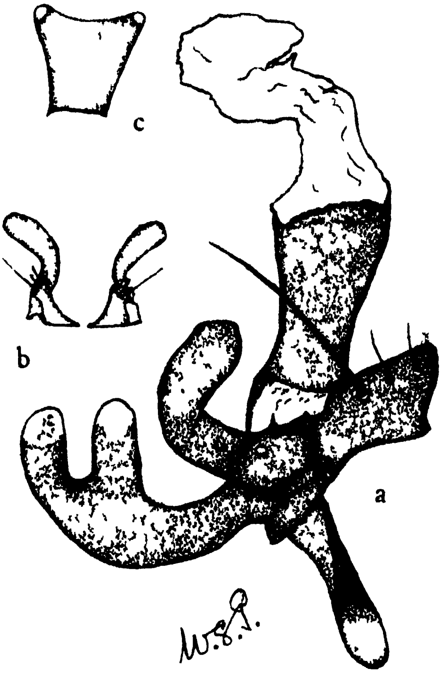

Fig 17 *a*—Phallosome and one paramere of *M mesopotamiensis*, *b*—Front view of parameres of same, *c*.—Front view of posterior process of phallosome of same from low angle (forking not well seen)

tergum 5 mainly bluish-grey with a distinct dark grey median area. Sterna dark brown to black.

Early Stages. Oviparous, egg without stalk, laid in patches of cow dung in the field. Third Larva. Anterior spiracles with eight to nine finger-like openings. Posterior Spiracular Plates. Fig. 18, *b*. Very large and densely chitinised, spiracular slits, especially the upper and lower, long, plates separated by about one-third width of plate.

Notes. This species is very abundant in Middle Iraq, especially along the banks of the Euphrates and Tigris. It may be seen in large numbers on cattle, horses, donkeys and dromedaries, following the biting flies, especially gad-flies (Tabanidae), *Stomoxys* and *M. crassirostris*, and feeding on any blood and serum which exudes from their bites; it swarms about the eyes and nostrils of animals, and causes much worry. It disappears in November to re-appear in March; the winter is passed in the puparium, numbers of which may be found buried in the earth around old patches of cow dung.

This species is of peculiar interest, for, although it is not a bloodsucker, the prestomal teeth are sufficiently well developed to scratch off a scab or enlarge a small wound; it is the only species in this group with this type of prestomal teeth known to me at present. Its proboscis has been described recently (1933).

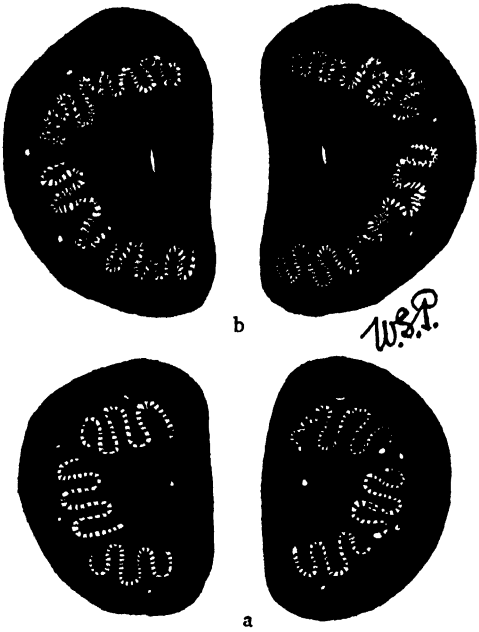

Fig. 18. *a.*—Posterior spiracles of third larva of *M. autumnalis*; note absence of distinct peritreme and dense nature of chitin of plates; *b.*—Same of third larva of *M. mesopotamiensis* (same scale).

14. ***Musca xantholemas** Wied. Auser. Zwei. Insekt. II, 54, 416, 1830.

Synonyms: *alba* Mall.; *albomaculata* auct. nec. Macq.; *convexifrons* auct. nec. Thomson.

Male. Head. Vertex linear, lower part and cheeks silvery; large facetted area. Mesonotum. Ground colour steel blue with four broad black stripes, which may appear in some dark specimens to fuse to form a single strip on each side. Abdomen. Orange with silvery spots and narrow dark bands; terga 1 and 2 dark brown to black, or only anterior half or so brown, remainder dark orange with some silvery sheen; tergum 3 orange, with a broad median black stripe spreading out T-shape along anterior border, and sometimes along posterior border, some silvery spots, or even stripes, bordering the median stripe, margins silvery ; tergum 4 very similar' but with a much narrower median dark stripe, which extends along posterior border forming a narrow dark band ; tergum 5 mainly dark with some silvery sheen.

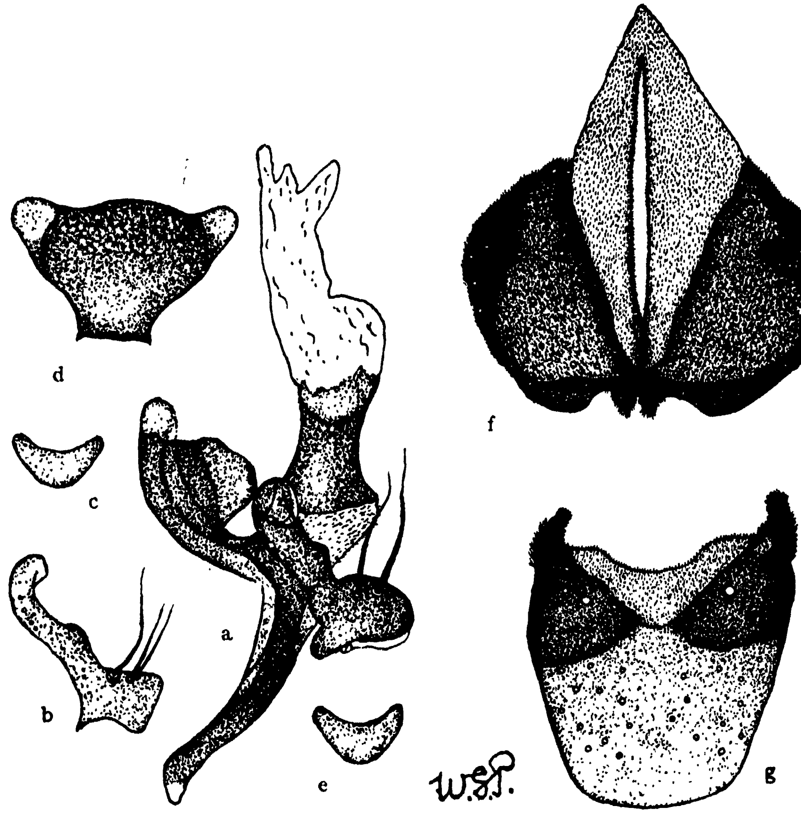

FIG. 19. *a*.—Phallosome and one paramere of *M. xanthomelas* ; note the long bent posterior part of paramere and hood-like posterior process ; *b*.—Side view of paramere of same ; *c*.—Front view of posterior process of phallosome of same ; *d*.—More enlarged view of same from behind ; *e*.—Another view of same from front ; *f*.—Anal cerci of same ; *g*.—Fifth sternum of same.

MALE TERMINALIA. Fig. 19. ANAL CERCI. Free borders emarginated near inner ends which are produced to form prominent nipples, outer ends broadly rounded. PHALLOSOME. Chitinous part short, about half length of membraneous portion ; posterior process broadly expanded hood-like, and forking rather shallow. PARAMERE. Anterior part characteristically short, with two to three rather long bristles ; posterior portion broad and bent, with one hump about the middle and with the usual sensory spines near the distal end. Fifth Sternum. Long, dark brown at distal end, posterior processes stout, projecting well, and serrated.

Female. Head. Vertex and cheeks silvery, almost (more than three-quarters) width of an eye ; vertical stripe about half width of vertex ; outer vertical bristles in two rows. Mesonotum. As in male, but if anything ground colour lighter. Abdomen. Orange with silvery spots and patches and dark posterior bands ; terga 1 and 2 light orange to dark brown ; if light orange, often without a median dark stripe, but only a small dark posterior band on each side of middle line ; if darker, commonly with a black median stripe and short posterior dark band ; tergum 3 either light or dark orange with a narrow median dark stripe, and nearly always with a well-marked narrow dark posterior band, admedian and marginal silvery patches or stripes ; tergum 4 similar, but dark posterior band usually more marked ; tergum 5 orange with some dark markings.

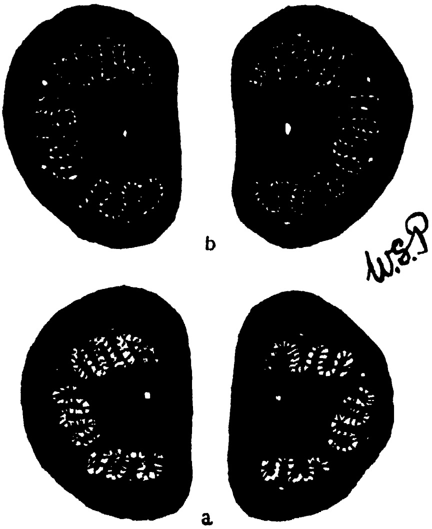

Fig. 20. *a.*—Posterior spiracles of third larva of *M. xanthomelas* ; *b.*—Same of third larva of *M. hervei* (same scale).

Early Stages. Oviparous, egg with a rather delicate long stalk, deposited singly, as are those of all species with this type of egg, in patches of cow dung in the field, the stalk being uppermost. Third Larva. When mature, greyish-white with a reddish-yellow dorsum. Anterior spiracles with seven to eight finger-like openings. Posterior Spiracular Plates. Fig. 20, *a.* Large, densely chitinised, plates separated by about one-fourth width of a plate. PUPARIUM. White.

NOTES. *Musca xanthomelas* is common in many parts of Egypt and the Sudan, especially in Khartoum and neighbourhood (Cottam). It is a haematophagous species, and is seen only on animals and on and about cow dung. It is widely distributed in Africa and all over India, where I have bred it in large numbers. It is not a very easy species to identify. The linear vertex of the male, together with the strikingly silvery vertex and cheeks of both sexes, especially well seen in fresh specimens, the bluish-white thorax, the orange abdomen, silvery spots and the dark posterior bands, especially on terga 3 and 4, are the important characters to note.

15. ***Musca hervei** Villeneuve. Ann. Sci. Nat. (Zool.). 10, 5, 335, 1922.

MALE. HEAD. Vertex linear, silvery ; cheeks silvery ; eyes minutely and scantily haired. MESONOTUM. Bluish-grey, posterior part and scutellum greyish-yellow with four broad black stripes. ABDOMEN. Mainly orange with silvery and yellowish-white markings ; terga 1 and 2 black with a small round silvery spot near middle of posterior edge of terga at middle of posterior border, posterior edge of terga often brown ; the light spots are occasionally wanting ; tergum 3 orange with a broad black median stripe bordered on each side by a silvery, or yellowish, patch which varies in size, remainder of tergum orange, and margins silvery ; tergum 4 very similar, but median stripe narrower, and posterior border with a dark band of varying width, light patches more yellowish ; tergum 5 almost entirely creamy, with suggestions of a dark brown admedian stripe. Sterna and adjoining edges of terga black (fig. 6).

MALE TERMINALIA. Fig. 21. ANAL CERCI. Very similar to those of *xanthomelas* (cf. figs. 19 and 21), nipples on inner ends, however, distinctly longer and stouter. PHALLOSOME. Chitinous part short and broad, shorter than membraneous part ; posterior process long, widely expanded, with shallow forking. PARAMERE. Anterior part long, with one long and two shorter bristles ; posterior part long, stout, tapering somewhat to end, which is bent. FIFTH STERNUM. Relatively small, black throughout, with rather narrow, inwardly-projecting processes.

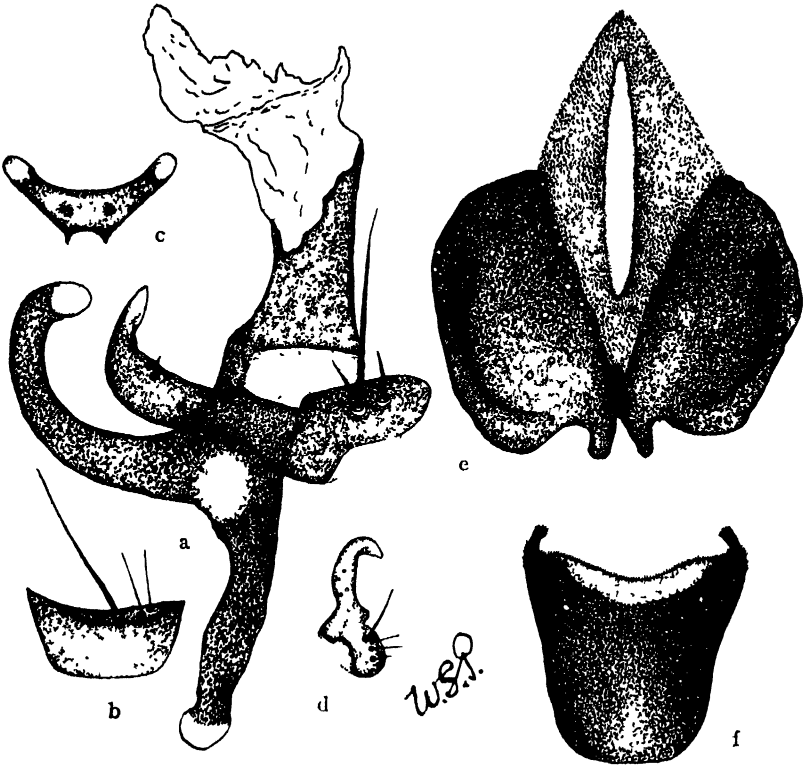

FIG. 21 *a*.—Phallosome and one paramere of *M hervei*, note the stout posterior part of paramere ; *b*.—Side view of anterior part of paramere of same ; *c*.—Front view of posterior process of phallosome of same ; *d*.—Side view of paramere of same ; *e* Anal cerci of same ; *f*. Fifth sternum of same.

FEMALE. HEAD. Vertex silvery, about three-quarters width of an eye ; vertical stripe about half width of vertex ; outer vertical bristles in two, sometimes in three, rows ; eyes minutely and scantily haired. MESONOTUM. Greyish or greyish-yellow, anterior end darker, as in male, with four black stripes. ABDOMEN. Black with white or yellowish-white spots and stripes ; terga 1 and 2 as in male ; tergum 3 black with broad white or yellowish-white stripe on each side of median black stripe, and silvery patches at margins ; tergum 4 similar, but median dark stripe narrower ; tergum 5 almost entirely yellowish or satiny, with dark lateral marks which vary in size ; sterna and adjacent margins of terga not as in male, but yellowish or creamy.

EARLY STAGES. Oviparous, eggs laid in patches of cow dung in the field. THIRD LARVA. Anterior spiracles with seven to eight finger-like openings. POSTERIOR SPIRACULAR PLATES. Fig. 20, *b*. Large, densely chitinised, slits very narrow and long, plates separated by about one-third width of a plate. PUPARIUM. Light, often greyish-brown.

NOTES. *Musca hervei* was recorded by Villeneuve from Tonkin and Shantung. It is widely distributed in North and Central China, and extends into Burma and North India. It is a haematophagous species, and is common on and about cattle and horses and cow dung. The male can be easily recognised by the minutely haired eyes, by the characteristically coloured orange abdomen with yellow and white spots, and by the strikingly marked sterna and adjacent edges of the terga. The female on the other hand is very like that of *convexifrons* and *autumnalis*. The minutely haired eye is a useful diagnostic character.

16. **Musca autumnalis** de Geer. Insek. VI, 83, 12, 1776.

SYNONYMS : *continua* R-D. ; *floralis* R-D. ; *grisella* R-D. ; *ludifacies* R-D. ; *nigripes* Panz. nec. Fab. ; **prashadi* Patton and Senior-White ; *rustica* R-D. ; *tau* Schrank.

MALE. HEAD. Vertex linear, lower part silvery ; cheeks white. MESONOTUM. Ground colour bluish-grey with four broad black stripes ; wing with three to four long hairs on upper side of R at base, never any bristles on ventral side of R_{4+5} to bend of M_{1+2}. ABDOMEN. Dark orange with a reddish tinge ; terga 1 and 2 dark brown to black ; tergum 3 dark orange with a broad median black stripe spreading out anteriorly to form a rather wide dark brown to black band, a small silvery patch on each side of anterior end of median stripe ; tergum 4 dark orange with a broad median dark stripe edged on each side with a yellowish-white stripe, yellowish-white patches at margins ; tergum 5 dark patch in middle and lateral yellowish-white stripes and marginal whitish spots ; sterna 1 and 2 and distal end of 5 dark brown.

MALE TERMINALIA. P. I, fig. 25 ; fig. 22 this paper. ANAL CERCI. Very similar to those of *hervei* and *xanthomelas*, if anything more deeply emarginated near inner ends, and nipples shorter, outer ends similarly broadly rounded. PHALLOSOME. P. I, fig. 25, *a*. Chitinous part varying in length, may be very short, as shown in illustration, or distinctly longer (particularly noted in specimens from Palestine) ; posterior process broadly expanded hood-like and well Y-forked. PARAMERE. Anterior part long and deep, with three to

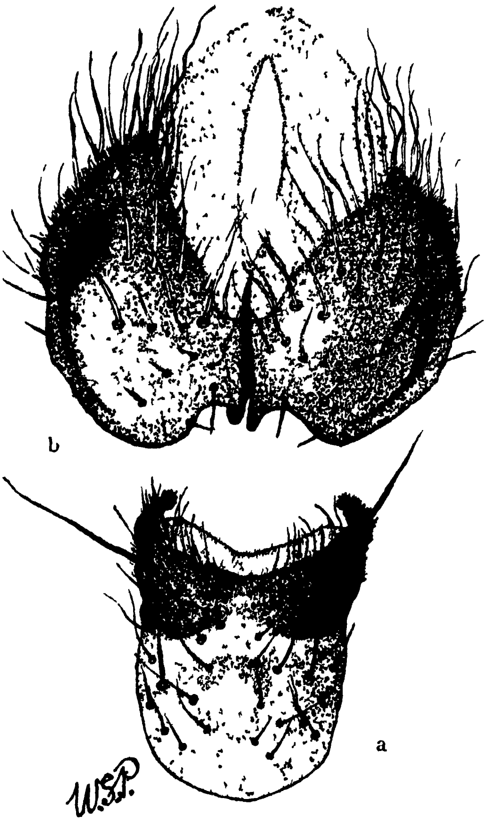

FIG. 22. *a.*—Fifth sternum of *M. autumnalis* ; note the rather long posterior processes, and compare with those of *larvipara*, *b* —Anal cerci of same (compare with those of *larvipara*).

four bristles, one usually very long ; posterior part long, stout, expanded at end which is markedly bent forwards. FIFTH STERNUM. Long and rather narrow, distal end dark brown, processes wide, projecting well, and slightly bent inwards (cf. with fig. 13, *f*).

FEMALE. HEAD. Vertex grey, wide (almost width of an eye) ; vertical stripe half width of vertex ; outer vertical bristles strong, and arranged somewhat irregularly in two rows ; cheeks grey. MESONOTUM. Ground colour slate grey with four broad black stripes ; wing pilotaxy as in male. ABDOMEN. Black with grey and white markings ; terga 1 and 2 black with two bluish-grey spots on posterior border on each side of middle line ; tergum 3 with a broad median black stripe, a wide grey stripe on each side, a large, somewhat triangular, dark admedian patch, and marginal grey patches ;

tergum 4 very similar, but median dark stripe narrower ; tergum 5 greyish with some dark patches.

EARLY STAGES. Oviparous, egg with long strong stalk, laid singly in patches in cow dung in the field. THIRD LARVA. Anterior spiracles with eight to nine finger-like openings. POSTERIOR SPIRACULAR PLATES. Fig. 18, *a*. Large, densely chitinised, spiracular slits not very long but fairly narrow, plates separated by about one-quarter width of a plate. PUPARIUM. White.

NOTES. *Musca autumnalis* is a widely distributed European species ; it extends down into Palestine, is common in Kashmir, and one male was taken at Tsinan, Shantung, North China. It is haematophagous in habit, is common on and about animals and cow dung ; it hibernates in this country in the adult stage.

I have not included *M. tempestatum* in this revision, for although it is common in parts of Africa, for instance at Marsabit, N. F. Province, Kenya (Mr. Symes), and also further north, I have no specimens from the region. I have also not included *M. gymnosomea* Rondani as, although I have specimens named by the late Professor Bezzi, I am not sure of its identity ; it was described from Malta. I hope some one in that island will give me an opportunity of examining specimens of *Musca* to see if it is possible to settle its identity. The final revision of the species of this region will be published in Part 3 of my book.

I wish to thank Mr. R. C. Shannon and Mr. G. Pandazis for a long series of fresh specimens of *M. larvipara* and *M. vitripennis* collected in Greece ; also Professor H. C. Efflatoun Bey for specimens of *M. albina*, *M. lucidula* and *M. vitripennis* from Egypt.

In my next paper in this series, I shall revise the species of the Ethiopian region.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

IV. ON THE SPONTANEOUS DISAPPEARANCE OF FLAGELLATES FROM AN INFECTED GLOSSINA

BY

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(*Received for publication* 22 *May*, 1933)

Research into the transmission of trypanosomes by Glossina proceeds inevitably along certain fixed lines. Clean tsetse are first fed upon animals infected with the trypanosome under investigation ; the flies are then nourished for a suitable period on a clean vertebrate ; and, finally, their infectivity is estimated either by feeding them on a clean animal or by dissection or by a combination of both methods.

Since Kleine's original work in 1909, the experience of a number of investigators, spread over many years and covering a very large amount of material, has, on the whole, confirmed his pronouncement that a fly is not infective until the salivary glands are invaded by the trypanosome. In the overwhelming majority of successful transmission experiments, in which dissection is relied upon to verify the results, one or more flies will be found infected in both alimentary tract and salivary glands, and to these flies the infection of the clean animal is ascribed. Where the investigation involves the transmission of *T. rhodesiense* or *T. brucei* by one of the game tsetses, several infective flies will usually be found, any one of which is capable of transmitting the trypanosome. On the other hand, in experiments with *T. gambiense* and *G. palpalis* the number of infected flies is often very small : it is by no means uncommon to find only one or two gland-infected tsetse in several hundreds employed in the experiment, and if these individuals are overlooked on dissection there will be nothing to account for the infection of the clean animal. There are, moreover, strains of trypanosomes very feebly, or not at all, transmissible by Glossina, which, nevertheless, still retain considerable power of development in the intestine of the fly. With a strain of this kind, in spite of a number of heavy gut infections in flies of mature age, no cyclical transmission will occur unless the salivary glands become invaded. The interpretation of experiments with such strains depends very largely on the dissection of the flies.

The significance of invasion of the salivary glands by a trypanosome has also been studied by the method of injection into a clean animal of the flagellates contained in the fly's intestine, after first removing the glands intact by careful dissection. By this method Yorke and his colleagues in Rhodesia obtained evidence suggesting that in certain circumstances forms infective to mammals are present in the intestine as well as in the glands of the fly. Bruce and his colleagues and their successors in Uganda failed, however, to confirm this observation which they believed to be explained either by leakage or, more probably, by deglutition of gland flagellates in the living fly. The fact that all Yorke's experiments were performed with flies whose glands were infected lends some force to this explanation and until it is proved that the intestinal flagellates of a fly whose glands are *not* infected can, on injection into a suitable animal, produce infection, there seems to be no need to qualify Kleine's original view that the gland forms are the sole infecting agents in the biological cycle.

There is, however, another point of importance in the conduct of transmission experiments. Experience has taught that, in normal circumstances, a fly once infected with a trypanosome of the polymorphic group remains infected all its life. Moreover, the evidence tends very strongly to show that once its glands have been infected, a fly will thereafter infect every susceptible animal it bites. In other words, when a fly has acquired an *infection totale* of gut and salivary glands, flagellates will normally persist in both gut and glands until the insect's death.

An exception to this general rule will only be detected when the infectivity of the flies is tested both by feeding them on a clean animal and by their subsequent dissection. If, in a batch of tsetse that has infected an animal, none is found on dissection to contain any flagellates, the possibility must be entertained that the trypanosome has died out in the fly or flies responsible for the infection. In these circumstances, if during dissection both the salivary glands and the intestine of all the flies have been examined, the disappearance will be held to be complete. If, however, the glands are only examined in flies whose intestine has first been found to be infected, there still remains the possibility that the gland flagellates have persisted while those of the gut have disappeared.

Some years ago this matter was discussed in connection with the failure to find a gland-infected fly in a batch that was known to have infected a monkey (Duke, 1921). It was then pointed out that, before explaining this result by supposing that flagellates had died out in a fly in which they were at one time established, there were certain simpler or more probable alternative explanations to be considered. The infective fly might have been overlooked until it had become too dry for dissection ; or, again, casual infection from some source outside the experiment might have taken place -the bane of this kind of investigation, and one that demands constant vigilance and careful control. In considering these alternatives, reference was made to the results obtained by feeding infective flies on monkeys whose blood contained recently administered arsenic, a procedure which caused the disappearance of the flagellates from the intestine of the fly, leaving those in the salivary glands unaltered in shape and function. There are, however, recorded observations which suggest that flagellates may indeed disappear from the gut, and possibly also from the glands of an infective fly. Bruce and his collaborators in Uganda described a transmission experiment wherein 16 laboratory-bred *G. palpalis* were fed on infected monkeys for 5 days and become infective 34 days later, remaining so for at least 49 days. ' On the 56th day the remaining 12 flies were dissected and examined for flagellates. None were found, but the contents of the alimentary canals of the 12 flies, pooled and injected into a healthy monkey, gave rise to Sleeping Sickness ' (Bruce *et al*, 1911). In this experiment the glands of the flies were not examined, so that we may presume that the flagellates had persisted in the glands after their disappearance from the intestine.

Later, in Nyasaland, working with *T. rhodesiense* and *G. morsitans* Bruce again met with a similar experience. In an experiment three infected flies were found. ' The first and second had the alimentary tract swarming with flagellates, but none in the salivary glands. The third was found, on dissection, to be free from trypanosomes throughout. This is curious, because this fly had been isolated in a glass tube as an infective fly, and had, when used alone on a rat and rabbit, infected both these animals. The fly remained alive in the tube for 13 days, and the only explanation that can be given is that in this case the trypanosomes disappeared absolutely from the fly some few days before its death. This was the first time this had been observed to take place, and it was thought to be a remarkable phenomenon and difficult to credit, until another example of the same kind was observed. It must, therefore, be held as probable that an infective fly, with presumably both salivary glands and alimentary tract swarming with trypanosomes, can lose all these flagellates and become non-infective ' (Bruce *et al.*, 1915). If we accept the implication that the rat and the rabbit were protected from casual infection (in the circumstances a legitimate assumption), there is little doubt that the authors' explanation is correct. The experience now to be described tends to confirm the accuracy of their conclusion.

Experiment 1316 comprised a batch of laboratory-bred *G. palpalis* fed for 2 days on a monkey infected with *T. gambiense* and then nourished for some 16 days on a clean fowl before being transferred to several clean animals, all of which in course of time became infected. On the forty-third day of the experiment the flies remaining alive were killed and dissected. In 1 male and 3 females flagellates were swarming in both gut and salivary glands. These 4 flies were all alive when the batch was exposed to chloroform. Another male fly, taken from the box dead, before its companions were chloroformed, revealed on dissection a state of affairs never before recorded in ordinary transmission experiments, but reminiscent of the effects of feeding infective flies on arsenic-containing blood.

The fly had died a natural death and had not fed for some days previous to its death, circumstances which of themselves normally exert no visible effect on flagellates in either gut or glands. The intestine was quite empty of blood and contained only a little pale straw-coloured fluid. Throughout the alimentary tract there were no flagellates, alive or dead, and no vestiges thereof. Both glands were crammed with flagellates, all of which were motionless and apparently dead though unaltered in form. As already observed, the death of the fly overnight cannot alone be held responsible for this remarkable phenomenon : it is a common experience at Entebbe to find swarms of living flagellates in both gut and glands in flies that have died overnight, even after several days of starvation, and this particular fly was alive the day before its dissection. As already stated, during the investigation of the action of arsenphenylglycin on trypanosomes, several infective flies were examined in which the arsenic had sterilised the gut of all flagellates, those of the glands being left intact and the fly remaining infective. The observation now recorded suggests, therefore, that a trypanosome infection may indeed die out from the alimentary canal in Glossina. The absence from this fly of any trace of dead flagellates suggests that the gut had been clear for at least 3 or 4 days previous to its death, as in the flies sterilised by arsenic dead and altered flagellates in process of disintegration were discernible for at least 2 or 3 days after the sterilising feed. It is probable that the flagellates in the glands of this fly had died before its death, and that they would eventually have disappeared, either by absorption or ejection, had the insect survived for a sufficient period after their demise. There would then have remained no recognisable sign of the previous heavy infection of this fly with flagellates in both its gut and salivary glands.

The fact that the phenomenon here recorded has never before been demonstrated by actual dissection testifies to the rarity of its occurrence. At the Entebbe Laboratory, in normal circumstances a fly such as that just described would be overlooked, owing to the absence of flagellates from the intestinal tract. But in a large number of special experiments both the salivary glands and the intestine have been dissected and examined in every fly ; and yet, until the present occasion, I have never found the glands infected without finding flagellates in the alimentary tract of the same fly.

It is, therefore, highly probable that the phenomenon is of very rare occurrence in the ordinary conditions of experiment or in nature. Nevertheless, the possibility of its occurrence must be borne in mind by those concerned with trypanosomiasis research.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

V. ON THE EFFECTS OF TEMPERATURE

BY

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The earliest published work on the effect of temperature on the development of trypanosomes in Glossina was carried out during 1911-12 by the Luangwa Sleeping Sickness Commission, working in Northern Rhodesia (Kinghorn *et al*, 1913). The tsetse in the Luangwa Valley was *G. morsitans*, the trypanosomes were *T. rhodesiense* and its allies in the game. The Commission studied the infectivity of the trypanosome to tsetse, both at Nawalia (2,100 feet) and at Ngoa (4,400 feet), on the Congo Zambesi Watershed, some 70 miles distant. It was found that the infection rate in wild tsetse in the valley was two and a half times that in the plateau flies. Moreover, in three series of experiments carried out at laboratory temperatures at Nawalia (75° to 84° F.), at Ngoa (59° to 65° F.) and at the latter place with the aid of the incubator (84° to 86° F.), it was shown conclusively 'that comparatively high temperatures of 75° to 85° F. are necessary for the completion of the developmental cycle of *T. rhodesiense* in *G. morsitans*.' The Commission's field and laboratory observations on the effects of temperature culminated in the following striking experiment. Two batches of *G. morsitans*, A and B, after feeding for three successive days on a guinea-pig infected with *T. rhodesiense*, were both kept at a mean laboratory temperature of 60° F. during the ensuing 36 and 57 days respectively, and fed regularly on a clean monkey. This monkey never became infected. On the fortieth day of the cycle in batch A, and on the sixty-first day in batch B, the flies were placed in the incubator for 6 to 8 days at 75° to 85° F. It was then found that the cycle had been completed in both batches. Batch A infected a clean animal on the forty-eighth day of the cycle, and in batch B a fly was found with infected salivary glands. This experiment is convincing. With batch A, it might be objected that the cycle in the flies was, perhaps, normally of some 40 days' duration, and was, therefore, just approaching completion at the time when the flies went into the incubator. But the fact that batch B failed to infect up to the sixty-first day of its cycle definitely disposes of this contingency, which with *T. rhodesiense* is in any case exceedingly remote. The authors conclude that ' whereas the earlier stages of the cycle may take place at comparatively low temperatures, such as 60° F., for the invasion of the salivary glands, higher temperatures, such as 75° to 85° F., are necessary.' Relative humidity was found to be unimportant, and the final conclusion was that the only factor which could explain their results was temperature.

The incubator temperatures employed in the Commission's investigations did not exceed 85° F. The climate at Ngoa, where the effects of the lower temperatures were studied, is considerably colder than that of Nigeria or Uganda, where, during the last few years, the subject has again been investigated on a considerably larger scale. In neither of the latter countries do laboratory conditions embrace the prolonged exposure of the flies to temperatures as low as 60° F. On the other hand, as will be seen, the upper range of temperature is extended considerably, either by the aid of the prevailing climate or by the incubator kept at from 90° to 98° F.

In 1912-13, Bruce and his colleagues (Bruce *et al.*, 1915) employed the incubator in their transmission experiments with *T. rhodesiense* and *T. brucei* in Nyasaland, using a temperature of 84° F. There are indications in the schedules that the higher temperature favoured development in the fly, but their work did not deal specifically with the effects of temperature.

In 1929, Lloyd in Nigeria (1930) placed a batch of 15 laboratory-bred *G. tachinoides* in the incubator at a temperature of 92° to 97° F. for the first 8 days of the cycle, and found on dissection that 86·6 per cent of the flies were infected. An attempt to reproduce this result in a second experiment, conducted under apparently identical conditions, yielded only 8 per cent. infections.

Taylor (1932) has recently carried out investigations on a much larger scale. He examined the transmissibility of 26 different strains of *T. gambiense* in *G. tachinoides*, and compared the effect of ordinary laboratory temperatures with that following exposure of the flies during their 4 days' infecting feeding to a temperature in the incubator of 98° F. Five thousand nine hundred and fifty-four flies were employed in the laboratory temperature experiments and 2,246 in the incubator series. In all the strains tested, Taylor found that there was an increase in the infection rate among the flies kept in the incubator. An analysis of his Tables shows that in the nine strains subjected to both laboratory and to incubator temperatures the greatest effect was observed in the two strains whose transmissibility by *G. tachinoides* at laboratory temperatures was lowest. In one of these, Gadau XIII, the ' total infectivity rate ' was increased from 2·9 to 20·8 per cent., and the ' cyclical development rate ' from 2·4 to 17·5 per cent., i.e., approximately a 7-fold increase. In the other, Ayu XI, the rises were from 1·5 and 1·5 to 16·0 and 11·9 per cent., i.e., approximately 11- and 8-fold increases. But the most remarkable record of them all is the behaviour of Strain Ayu V, for which the figures were 10·5 and 9·8 per cent. at laboratory temperature, and 77·9 and 73·5 per cent. in the incubator experiments. This strain was the most transmissible of all the 26 tested under laboratory conditions. Maintenance in the incubator for a longer period than the four days of infecting feeding apparently produced no additional effect.

There are several points arising out of Taylor's interesting paper which will be discussed in later publications in this series and elsewhere. For the present our concern is only with the effect of temperature. It is plain from the text of his paper that Taylor accepts Robertson's views about the endogenous cycle of the polymorphic trypanosomes in the vertebrate host and the influence exerted by this cycle on the daily infection rate of tsetse serving in transmission experiments (Robertson, 1913). Thus he writes : ' It is hardly necessary to point out that temperature is only one of several factors, all of which play more or less important parts in determining infection rates. A most important factor is the type of trypanosome taken up by the fly, this being dependent on the stage of the endogenous cycle in the animal from which the tsetse is infected. This factor is responsible for the great fluctuations in infection rates in batches of tsetse fed over a long period on an infected animal ' (p. 414) ; again : ' It is essential in these tests to employ large numbers of flies fed on the strain over a reasonably long period in order to strike an average of the infection rates, which vary so much at different periods of the endogenous cycle in the vertebrate ' (p. 409). And yet there is no indication that Taylor's experiments are paired so that ' hot ' and ' cold ' batches of tsetse were fed on the infecting animal simultaneously. In a later paper of this series, data will be submitted showing how great may be the diurnal variations in the infection rate, especially in experiments with readily transmissible strains. Another point apparently disregarded in the planning of Taylor's experiment is the possible effect on the transmissibility of *T. gambiense* of prolonged sojourn in one and the same animal. It has been shown, with *T. gambiense* and *G. palpalis*, that it is misleading to treat as identical the transmissibility of a strain at the commencement and at the end of its career in the same vertebrate host (Duke, 1931).

Taylor's Strain Gadau XIII was under examination in the ' laboratory ' series from January to May, and in the ' incubator ' series from January to March, during which time it was kept in 5 guinea-pigs, representing the third to the seventh passages. Similarly with others of the 9 doubly-tested strains. The data about Strain Ayu V, the most spectacular of all these tested, emphasize the desirability of a more rigid comparison. The period during which this strain was under examination extended over 102 days, from May to August, during which time the flies were fed on two monkeys, representing the first and second passages of the strain from man. Over this period, only 143 flies were used in the ' laboratory ' and 117 in the ' incubator ' series. According to the number of flies per batch, these totals probably each represent 3 or 4 batches of flies, each batch being put on the infecting animal for 4 successive days. Strain Ayu V is the most transmissible of all the Taylor's strain, and in 25 per cent. of infections in flies dissected on the thirteenth day after the first infecting meal the salivary glands were already invaded, in the ' laboratory temperature ' series. According to my experience, both with *G. morsitans* and *G. palpalis*, it is just these readily transmissible strains that show the greatest diurnal and periodic variations in their infectivity to fly. In such circumstances the different phases of the endogenous cycle may alone account for considerable differences in the infection rates of the two groups of flies.

But, when all is said, the relatively enormous infection rate of 77·8 per cent. attained by Strain Ayu V, taken in conjunction with Lloyd's record of 86·6 per cent. infected in 15 flies and Taylor's observation that in certain individual batches of flies 100 per cent. were infected (p. 411), constitutes irrefutable proof of the soundness of Taylor's contention that the higher temperatures favour the development of *T. gambiense* in *G. tachinoides*. Whether or not this conclusion can be extended to *T. gambiense* and *G. palpalis* and, more widely still, to the polymorphic trypanosomes and tsetse generally, is another matter, upon which there will be more to say later on.

Before passing on to consider the experimental evidence accumulated during the last two years at Entebbe, it may be remarked that the behaviour of Taylor's Gadau and Ganawuri groups of strains at laboratory temperatures, as set forth in his Table I, falls well within the limits of my own experience of *T. gambiense* in *G. palpalis* at Entebbe. The figures for his Ayu group of strains, on the other hand, are strongly reminiscent of the manner in which *T. rhodesiense* behaves in *G. palpalis* under the conditions obtaining in the Entebbe Laboratory. It is interesting also to note in the annual report of the Medical Department, Nigeria, for 1931, that some of the strains isolated from the Ayu district possess attributes recalling *T. rhodesiense*.

In the experiments to be described below it was found that, provided the humidity was maintained and air admitted to the incubator, the flies did fairly well. Flies that fail to feed well die rapidly in the incubator, and if the air or water supply fails every fly may die within 24 hours. In some experiments the flies, having had a full meal, were kept in the incubator without food for 48 hours, fed again and kept for a further 24 hours in the incubator at 95° to 98° F., without serious mortality. Life in the incubator is thus possible but precarious for the *G. palpalis* of Lake Victoria, and it was found that a very slight disturbance of the optimal conditions led at once to a serious mortality in the incubated flies. It must be remembered, however, that *G. palpalis* in Uganda is accustomed to climatic conditions very different from these described by Taylor for *G. tachinoides* in Nigeria, so that the contrast between life in the incubator at 95° to 98° F., and that in the laboratory or in the natural environment of the fly is very much greater in Uganda than in Nigeria. Reference to the Protectorate Blue Books shows that the absolute maximum temperature recorded at the Entebbe screen station (130 feet above the lake shore where *G. palpalis* lives) is 86° F. for both 1930 and 1931, the average maximum for 1930 being 81° F. and for 1931 82° F. The absolute minimum for each year was 59° F., the mean minimum for 1930 being 59·2° F. and for 1931 60·2° F. Of the 19 meterological stations scattered through the Protectorate there is only one, at Mbale in the Eastern Province, where an absolute maximum temperature of over 100° F. was recorded during 1930 and

Table I

Laboratory temperatures at the spot where the flies are kept at Entebbe

	Jan.		Feb.		March		Apr.	May	June	July		Aug.		Sept.		Oct.		Nov.		Dec.	
	1931	1933	1931	1933	1931	1933	1933	1933	1930	1930	1932	1930	1932	1930	1932	1930	1932	1930	1932	1930	1933
Maximum dry bulb	80	82	83	82	80	82	82	...	76	74·5	78	77	79	77	79	79	80	79	80	79	80
Minimum dry bulb	65	69	69	69	70	70	71	...	68	69	67	66	68	70	68	69	69	69	70	64	69
Minimum wet bulb	64	67	65	66	65	66	68	...	65	64	64	62	64	65	65	65	66	65	66	64	66
Mean dry bulb 8 a.m. ...	73·2	73·2	76·2	72·4	73	73	72·1	...	71	70·8	70·3	71·5	70·8	70·3	70·7	74·1	73·1	73·9	72·7	73·4	72·3
Mean dry bulb 12 noon ...	76·7	76·6	78·2	73·1	76	78	78·6	...	73·7	73·1	73·4	73·7	75	70·0	75·2	75·2	76·6	76·8	77·0	75·6	76·2
Mean dry bulb 4 p.m. ...	75·5	78·4	77·8	77·8	75·6	79·7	80·0	...	73·6	73·4	75·7	74·2	76·6	70·3	76·6	75·3	77·7	76·3	78·0	75·6	78·0
Greatest daily difference between dry and wet bulbs ...	11	8	10	9	7	10	10	...	9	9	9	11	10	9	9	10	9	12	10	11	10
Mean humidity 8 a.m. ...	70·7	...	77·3	...	81·6	...	...	...	78	76·5	78·9	76	80·1	77·8	84·9	76·6	81·1	78·6	79	75·6	82·4
Mean humidity 12 noon ...	71·3	...	79·0	...	77	...	...	...	73	72·6	73·5	72·6	71·1	74·6	76·2	73·1	72·7	74·6	71·1	70·4	73·4
Mean humidity 4 p.m. ...	71·4	...	79·2	...	78·8	...	...	...	70	73·6	70·1	71·1	65·2	74·4	73·8	70·6	71·3	75·5	70·1	71·4	73·1

1931, and this on one day only. 1930 and 1931 were normal years. In striking contrast to the mild and comparatively constant climate enjoyed by *G. palpalis* on Lake Victoria, Taylor records screen readings, taken in the shade at a primary centre of *G. tachinoides* at Gadau, showing mean maxima of 110·1° F. (43·4° C.) for March and 105·9° F. (41·1° C.) for April, 1931, during the hot season. The lowest temperature recorded at the Tsetse Investigation screens, Gadau, was 44° F. (6·5° C.) in January, 1932. The lowest mean minimum for a year's observation was recorded in January, 50·9° F. (10·5° C.). The alternation of hot and cold seasons that forms so striking a feature of the Nigeria records does not take place in Uganda. Table I shows the laboratory temperatures at the actual place where the flies are kept. The 1930-31 records in this Table were taken at the Veterinary Laboratory, Entebbe ; those of 1932-33 at the present Human Trypanosomiasis Institute building.

A table of equivalent temperatures on the three scales is given for the convenience of readers.

Fahrenheit	Réaumer	Centigrade	Fahrenheit	Réaumer	Centigrade
65	14 7	18·3	82	22·2	27 8
66	15 1	18 9	83	22 7	28 3
67	15 6	19·4	84	23·1	28·9
68	16·0	20·0	85	23·6	29·4
69	16·4	20·6	86	24·0	30 0
70	16·9	21·1	87	24·4	30·6
71	17·3	21·7	88	24·9	31·1
72	17·8	22·2	89	25·3	31·7
73	18·2	22·8	90	25·8	32·2
74	18·7	23·3	91	26·2	32·8
75	19·1	23·9	92	26·7	33·3
76	19·6	24·4	93	27·1	33·9
77	20·0	25·0	94	27·6	34·4
78	20·4	25·6	95	28·0	35·0
79	20·9	26·1	96	28·4	35·6
80	21·3	26·7	97	28·9	36·1
81	21·8	27·2	98	29·3	36·7

METHOD

Every test consisted of a pair of boxes of flies, each containing at the outset approximately 50 *G. palpalis*. The two boxes of each pair were fed, one immediately after the other, on the infecting animal.

One box was then placed in the incubator and the other over the shallow water-trays in the fly-room at the laboratory. The incubator contained large dishes of water which were refilled each morning. Both doors were left ajar to admit a free supply of air to the flies.

The experiments were divided into two series. In the first series, the incubator was maintained at 86° to 88° F.; in the second, at 95° to 98° F. In the column headed ' Infecting Feeds,' the number shows the days the flies fed on the infecting animal; *alt.* means that each feed was followed by 48 hours' starvation : *consec.*, that the flies were given an opportunity (seldom taken by all) of feeding daily for the period indicated. The length of exposure in the incubator is shown : *alt.* occurring in this column means that the box was kept in the incubator for the 24 hours immediately following the infective feed and then removed to room temperature for the following 24 hours' starvation, this procedure being repeated in connection with the second infective feed ; *consec.* means that the flies stayed in the incubator, except for the brief periods at meals, from their first infecting feed until 24 hours after their last. Thus every fly in the incubator series spent 24 hours in the incubator after each infecting feed ; some batches were kept there continually for the whole period of the infecting feeding ; others again were withdrawn for a period of 24 hours' starvation between two infecting feeds.

The experiments are grouped roughly according to strains and to the infecting animals on which the flies were fed. A summary of each group is given in the right hand columns. Unless otherwise stated, the infecting animal was a monkey (*Cercopithecus* sp.) ; *Sh.* = Sheep ; *Rab.* = rabbit ; *b.* = *T. brucei* ; *g.* = *T. gambiense* ; and *rh.* = *T. rhodesiense*.

Infected flies dying during the early days of an experiment, and which still contained the blood of the infecting animal, were rejected in both series.

In addition to the experiments presented in the following Table, a number of others were conducted with strains held to be non-transmissible, some of which had lost all power of developing in *G. palpalis* at Entebbe. In no instance did exposure to the incubator at 95° to 98° F. enable these latter strains to develop in the tsetse : all the flies, kept at either incubator or room temperature, remained entirely negative. It is not thought necessary to include the figures for these strains here.

TABLE II

The summaries of the transmission experiments. The components of each pair of experiments are bracketted together

SERIES I

(Laboratory temperature and incubator at 86° to 88° F.)

Experiment No.	Species of trypanosome and laboratory strain number	Infecting animal	Infecting feeds	Days in incubator	Flies dissected	
					Number	Containing flagellates
634	b. LIII	948	2 alt.	0	44	0
635	"	"	"	4 consec.	28	2
636	rh. T2	950	"	"	46	1
637	"	"	"	0	46	9
639	b. LIII	949	"	4 consec.	33	1
640	"	"	"	0	48	2
664	rh. T15	958	1 feed	25 consec.	44	0
665	"	"	"	0	50	2
674	rh. T3	923	2 alt.	21 consec.	50	3
675	"	"	"	0	52	2
689	g. 'Br.'	953	2 alt.	0	46	1
690	"	"	"	12 consec.	55	3
771	b. LIII	969	2 alt.	4	57	3
772	"	"	"	0	52	5
779	"	Sh. 51	"	12 consec.	42	0
780	"	"	"	0	49	2
783	"	969	"	12 consec.	28	0
784	"	"	"	0	52	1
785	"	"	"	12 consec.	44	4
786	"	"	"	0	53	3
796	g. 'Br.'	974	"	12 consec	31	0
797	"	"	"	0	44	0
802	b. LIII	Sh. 51	"	12 consec.	31	0
803	"	"	"	0	40	0
804	"	969	"	12 consec.	47	0
805	"	"	"	0	43	0
810	"	Sh. 51	"	12 consec.	27	0
811	"	"	"	0	50	0
822	g. 'Br.'	974	"	12 consec.	28	0
823	"	"	"	0	38	0
829	b. LIII	Sh. 51	"	12 consec.	38	1
830	"	"	"	0	45	0
833	"	"	,	12 consec.	17	0
834	"	"	"	0	37	0
858	g. 'Br.'	974	"	12 consec.	24	0
859	"	"	"	0	56	3
863	"	,	"	12 consec.	43	1
864	"	"	"	0	39	2
876	"	"	"	12 consec.	28	1
877	"	"	"	0	71	4
878	b. LIII	969	"	12 consec.	19	0
879	"	"	"	0	29	2
885	g. 'Br.'	974	"	12 consec.	14	0
886	"	"	"	0	54	2
891	b. LIII	969	"	12 consec.	43	9
892	"	"	"	0	38	0
m921	b. LIV	Rabbit B	"	4 consec.	30	2
m922	"	"	"	0	33	2

SERIES I.—*Continued*

Experiment No.	Species of trypanosome and laboratory strain number	Infecting animal	Infecting feeds	Days in incubator	Flies dissected	
					Number	Containing flagellates
m923	b. LV	981	"	4 consec.	28	2
m924	"	"	"	0	39	5
1005	rh. T1	1015	"	4 consec.	22	13
1006	"	"	"	0	44	0
1011	"	"	"	5 consec.	38	0
1012	"	"	"	0	51	2
1081	rh. T2	1025	1	0	46	0
1082	"	"	1	14	27	2
1085	"	"	2 consec.	0	22	0
1086	"	"	"	12	30	1
1088	rh.	1038	2 alt.	0	52	2
1089	"	"	"	29 consec.	24	1
1091	"	"	"	0	42	0
1092	"	"	"	11 consec.	23	0
1094	b. LIII	1026	"	0	50	1
1095	"	"	"	12 consec.	38	2
1097	T. brucei	1037	"	8 consec.	41	0
1098	"	"	"	0	42	0
1099	"	"	"	8 consec.	50	0
1100	"	"	"	0	53	0
1102	th. T2	Cat	"	6 consec.	33	1
1103	"	"	"	0	44	0

The data of the two series were subjected to examination by the formula $\chi^2 = \int\frac{(x - m)^2}{m}$.

For Series I (laboratory temperature and incubator at 86° to 88° F.), the value for $\chi^2 = 2{\cdot}2404$. In Series II (laboratory temperature and incubator at 95° to 98° F.), the corresponding value $= 5{\cdot}0391$.

These values indicate that, whereas the difference between the two samples in Series I may well be due to chance, in Series II the higher temperature is in all probability responsible for the greater infection rate shown by the incubated flies.

In assessing the significance of his results Taylor concludes that the increased infection rate in the incubated flies is due to the beneficial effect of the high temperature on the trypanosome, helping it in its early efforts at multiplication and establishment in the fly. Retention of the flies in the incubator after the period of infecting feeding was found to produce no further effect.

The experiments of Table II suggest that this interpretation alone is inadequate, and that the influence of the higher temperature on the fly itself must also be taken into account. It has been shown that *G. morsitans* from Tanganyika Territory is about twice as good a

TABLE II.—*Continued*

SERIES II

(Laboratory temperature and incubator at 96° to 98° F.)

Experiment No.	Species of trypanosome and laboratory strain number	Infecting animal	Infecting feeds	Days in incubator	Flies dissected		Totals per individual test					
							Incubator			Bench		
					Number	Containing flagellates	Number	Positive	Per cent.	Number	Positive	Per cent.
1226	b. LIII	1065	2 alt.	0	52	3	192	20	10·5	203	11	5·4
1227	"	"	"	2 alt.	53	2						
1232	"	"	"	0	36	1						
1233	"	"	"	4 consec.	52	8						
1221	"	1068	"	0	45	6						
1220	"	"	"	2 alt.	37	6						
1235	"	"	"	4 consec.	50	4						
1236	"	"	"	0	33	1						
1237	g. 'Br.'	1070	"	2 alt.	52	0	281	5	1·7	402	5	1·2
1238	"	"	"	0	45	0						
1242	"	"	"	2 alt.	64	0						
1243	"	"	"	0	57	1						
1244	"	"	"	4 consec	31	3						
1245	"	"	"	0	45	0						
1299	"	"	2 consec.	0	52	0						
1300	"	"	"	3 consec.	44	0						
1340	"	"	"	3 consec.	16	1						
1341	"	"	"	0	52	0						
1342	"	"	1	2 consec.	34	1						
1343	"	"	"	0	54	0						
1368	"	"	2 alt.	0	56	1						
1369	"	"	"	2 alt.	33	0						
1274	b LIII	1087	"	4 consec.	25	0	194	3	1·5	232	7	3·0
1275	"	"	"	0	45	0						
1276	"	"	1	2 consec.	35	1						
1277	"	"	1	0	36	1						
1279	"	1086	2 alt.	4 consec.	42	2						
1280	"	"	"	0	50	0						
1281	"	"	3 consec.	4 consec.	49	0						
1282	"	"	"	0	55	3						
1267	"	"	2 alt.	2 alt.	43	0						
1268	"	"	"	0	46	3						
1294	rh T1	1089	3 consec.	4 consec.	42	0	169	5	2·9	182	5	2·7
1295	"	"	"	0	40	1						
1307	"	"	"	0	46	0						
1308	"	"	"	4 consec.	47	3						
1331	"	"	2 consec	0	51	2						
1332	"	"	"	3 consec.	40	1						
1370	"	"	2 alt.	2 alt.	40	1						
1371	"	"	"	0	45	2						
1305	g. LI	1090	3 consec	0	39	1	153	0	0	180	3	1·6
1306	"	"	"	4 consec	31	0						
1312	"	"	2 consec.	3 consec.	33	0						
1313	"	"	"	0	43	1						
1321	"	"	"	0	55	0						
1322	"	"	"	3 consec.	47	0						
1344	"	"	1	0	43	1						
1345	"	"	1	2 consec.	42	0						

SERIES II.—*Continued*

Experiment No.	Species of trypanosome and laboratory strain number	Infecting animal	Infecting feeds	Days in incubator	Flies dissected		Totals per individual test					
							Incubator			Bench		
					Number	Containing flagellates	Number	Positive	Per cent.	Number	Positive	Per cent.
1301	g. LI	1092	1	0	54	0	192	8	4·1	211	1	0·4
1302	,,	,,	1	3 consec.	55	0						
1309	,,	,,	3 consec.	0	47	0						
1310	,,	,,	,,	4 consec.	44	5						
1319	,,	,,	,,	4 consec.	40	3						
1320	,,	,,	,,	0	58	0						
1337	,,	,,	2 consec.	0	52	1						
1338	,,	,,	,,	3 consec.	53	0						
1317	g. LII	1098	3 consec.	4 consec.	47	5	148	9	6·0	199	6	3·0
1318	,,	,,	,,	0	48	2						
1330	,,	,,	2 consec.	3 consec.	38	2						
1331a	,,	,,	,,	0	48	1						
1351	,,	,,	,,	3 consec.	20	5						
1352	,,	,,	,,	0	49	0						
1362	,,	,,	2 alt.	2 alt.	42	0						
1363	,,	,,	,,	0	55	0						
1315	,,	1095	2 consec.	0	50	1	111	7	6·3	155	3	1·9
1316	,,	,,	,,	3 consec.	29	5						
1326	,,	,,	,,	0	54	0						
1327	,,	,,	,,	3 consec.	44	2						
1335	,,	,,	,,	0	51	5						
1336	,,	,,	,,	3 consec.	38	0						
1353	rh. T1	1096	2 alt.	2 alt.	43	0	132	5	3·7	153	1	0·6
1354	,,	,,	,,	0	40	2						
1360	,,	,,	,,	0	52	1						
1361	,,	,,	,,	2 alt.	50	2						
1372	,,	,,	2 consec.	3 consec.	42	1						
1374	,,	,,	,,	0	58	0						
1357	,,	1105	2 alt.	2 alt.	32	1	81	4	4·9	108	5	4·6
1358	,,	,,	,,	0	59	2						
1364	,,	,,	,,	0	49	3						
1365	,,	,,	,,	2 alt.	49	3						
1378	?g. ?rh.	1113	,,	0	59	1	121	3	2·4	164	11	6·7
1379	,,	,,	,,	2 alt.	44	1						
1380	,,	1112	,,	0	57	4						
1381	,,	,,	,,	2 alt.	44	2						
1383	,,	,,	,,	,,	33	0						
1384	,,	,,	,,	0	48	6						

transmitter of man's trypanosomes as is the *G. palpalis* of Lake Victoria (Duke, 1933) ; and the work of Lloyd and of Taylor suggests strongly that *G. tachinoides* is in certain circumstances a still more efficient transmitter. If the higher temperature acted solely, or even mainly, on the trypanosome, surely the differences between the incubated and non-incubated samples in the Entebbe series would be greater than they actually are, for there is no reason to believe there is any fundamental difference between a representative series of strains of *T. gambiense* from Nigeria and one from Uganda in their response to temperature changes. If, on the other hand, the higher temperature acts through the tsetse by affecting its physiological processes in some way favourable to the trypanosome, then the difference between the two sets of experiments is more easily intelligible.

Taylor pertinently remarks that four days' continuous exposure to 37° C. is very different from the conditions met with by a fly in nature, even where the mean maximum temperature in the shade reaches 43·0° C. But this difference is not nearly so great as that between the incubator conditions and the normal environment of *G. palpalis* on Lake Victoria. The difference between the temperatures in the laboratory in Nigeria and those outside are, in certain important respects, very great, and the seasonal contrasts of the Nigerian climate, though reduced, are clearly reflected in the laboratory records. At Entebbe there is neither much difference between the temperatures in the laboratory and along the lake shore a few hundred yards away, nor are there pronounced seasonal variations throughout the year ; and it must be realised that *G. palpalis* on Lake Victoria never experiences anything approaching the rigours of climate to which *G. tachinoides* is accustomed in Nigeria.

These considerations suggest that the high temperature exerts its effect on the infection rate largely by the alterative action it has on the organisation of the tsetse. It is possible that the different species of tsetse all differ in their power of transmitting trypanosomes ; but we must assume that for the completion of the cycle in Glossina a temperature above a certain minimum is necessary, whatever the species of tsetse. Above this minimum requirement, higher temperatures may affect differently the different species of Glossina, as regards their power of transmitting trypanosomes. For a tsetse to transmit to the limit of its capacity it is probable that it must enjoy its optimum temperature. With *G. palpalis*, exposure to 36° to 37° C. causes a slight increase in efficiency, an increase that may, perhaps, represent the direct effect of the higher temperature on the blood trypanosomes during their first few days in the alimentary canal of the fly. To *G. tachinoides*, 37° C. is a temperature well within its normal experience in nature, and brings out to the full the latent capability of that species to transmit the trypanosome.

There is yet another consideration that supports this view. It is generally accepted that the behaviour of trypanosomes in culture on artificial media and in the preliminary stages of their development in tsetse is similar. A number of people have studied trypanosomes in the culture tube, and the great majority have found that temperatures between 18° and 30° C. are most favourable to development. Nöller, working with *T. theileri*, found that when his cultures were exposed to 37° C. the crithidia present at room temperature reverted to the blood trypanosome type (Nöller, 1920). The evidence afforded by culture experiments does not, therefore, support the view that the trypanosome itself benefits directly by the high temperature which it encounters in the incubator, although it may be retorted that the ordinary technique of culture is devised to imitate as far as possible the conditions in the insect.

This, then, is the interpretation suggested by a study of Taylor's results and those submitted in the present report. Exposure of Glossina, carrying *T. gambiense* or *T. rhodesiense*, to a temperature of 95° to 98° F. during the period of infecting feeding, tends to raise the infection rate of the flies. The increase may be due partly to the direct effect of the higher temperature on the trypanosome in the fly during its early struggles to establish itself, but it is mainly due to the effect produced on the vital processes of the tsetse, with the consequent reduction of the inhibitory action which the fly's tissues normally exert on the blood forms of the trypanosome as it strives to establish themselves in their intermediate host.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

VI. ON THE DURATION OF THE BIOLOGICAL CYCLE IN GLOSSINA

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There are two ways in which the time required for the completion of the biological cycle of a polymorphic trypanosome in Glossina can be estimated: by dissection and examination of the salivary glands of the flies at suitable intervals after the infecting feed, or by feeding them upon clean susceptible animals and calculating the date when infection of the animal actually took place. A combination of the two methods may also be employed. At the present time it is a generally accepted fact that a fly is not infective until the metacyclic forms of the trypanosome are established in its salivary glands. According to Robertson (1913), some 2 to 5 days after the first arrival of flagellates in the glands the bite of the fly becomes infective; and it has been observed occasionally at Entebbe that an animal which has been bitten by a fly with a very early infection of the glands has escaped infection.

To obtain accurate information by the method of dissection, batches of flies must be killed at intervals along the cycle, a procedure demanding large numbers of tsetse and readily transmissible strains for really satisfactory results.

The method of testing on a clean animal, together with dissection of all the flies as they die, is on the whole very satisfactory, provided that the climate is not too hot and dry. In Uganda this method yields good results; but in Nigeria, for example, flies dying over night are liable to dry up before morning. Taylor, commenting on this point, remarks of flies dying during the experiment that they are 'often rendered useless by very heavy bacterial infections'

(Wallace, 1930). Wallace's fifth conclusion reads :—' Evidence is given to show that tsetse flies do not pick up bacterial infection even from freshly contaminated surroundings.' He remarks that sometimes infections with a motile bacillus, quite distinct from the symbiots normally present in every fly, are found in tsetse that have died over night. Such infections are, however, very rare and a fly so infected is, of course, rejected. As an illustration of the rarity of this bacillary infection it may be noted that only a very few have been seen in the course of the examination of some 30,000 laboratory-bred flies dissected during 1932, and the majority were in flies that had been exposed to incubator temperature of 95° to 98° F.

Investigations carried out along these lines will reveal when the flies became infective. A batch of flies is kept alive for a sufficient time to allow of demonstration of its infectivity or otherwise, and is then dissected. By deducting from the date of the first appearance of trypanosomes in the test animal the number of days necessary for the incubation period in that animal, the date of the cycle at which the flies became infective can be determined. In an experiment of this kind it often happens that no flies with gland infections are found until the final dissection of the batch ; the earliest evidence of completion of the cycle will then be afforded by the infection of the test animal. On the other hand, a fly dying comparatively early in the experiment may show a gland infection on dissection and yet never have bitten the test animal.

It has been shown that a heavy infection with trypanosomes does no apparent harm to the tsetse (Duke, 1928) ; indeed, it is common to find that a large proportion of the total infected flies in a batch survives to the end of an experiment. In many experiments, especially those dealing with readily transmissible strains, the majority and sometimes all of the flies with gland infections survive until the final dissection of the batch. It is, however, well to remember that the glands of all these flies may actually have been infected days or weeks before their dissection. We have only to suppose that robust tsetse are at least as well suited to the trypanosome's requirements as more delicate individuals, in order to realise that flies with gland infections will tend to live through to the end of an experiment. And, as a corollary to this, if in a collection of flies devoted to a certain strain the first gland-infected fly is found on, say, the thirtieth day of experiment, the flies not being tested on a clean animal, then there is no justification for assuming that the completion of the cycle was delayed until the thirtieth day. The fly then dissected might have had its glands infected on the sixteenth, the twentieth or any other previous day of the experiment. It will be seen later on that these remarks are germane to the discussion that follows.

The biological cycle of *T. gambiense* and *T. rhodesiense* in different species of tsetse has been studied by various investigators in Africa. In the earlier work, the second of the methods was employed, viz., testing the flies on a clean animal.

With *T. rhodesiense*, and with some strains of *T. gambiense*, this method is, on the whole, fairly accurate, but with many strains of *T. gambiense* it is not. The incubation period of this latter trypanosome in monkeys may be very much longer than the 7 to 8 days usually allowed in these calculations. I have known a lapse of 3 weeks before *T. gambiense* appeared in the peripheral blood of a monkey inoculated by means of the syringe, although each day a stained thick film of its blood was examined. If examination is confined to ordinary fresh blood films the error will, of course, be still greater. When infection is conveyed by the bite of a tsetse, the incubation period in the animal may also be prolonged. In the Entebbe records, there are three excellent examples of the delayed appearance of trypanosomes in the peripheral blood of the test monkey, when stained thick films of its blood were examined daily. In each case the period is reckoned from the last day on which the infective fly bit the monkey, so that the estimate is conservative : with Strain XIV, 21 days ; with Strain XV, 39 days ; and with Strain IX, 25 days.

Readily transmissible strains often exhibit a fairly regular and short incubation period in the vertebrate, possibly because in transmission experiments with such strains there are often several heavy gland infections among the flies, which ensure a heavy inoculation of metacyclic forms. But with strains of low transmissibility there may be only one fly with a gland infection, and that light in character. The dissection method, if specially directed to this end, is undoubtedly the most accurate way of ascertaining the time taken for the completion of the cycle ; but, as already stated, its proper application demands large numbers of tsetse.

In the first part of this paper a review will be taken of observations bearing on the length of the cycle of *T. gambiense*, and, to a less extent, of *T. rhodesiense*, in different parts of Africa, including an account of 30 different strains of *T. gambiense* that have been studied at Entebbe since 1926.

In Part II are collected from various sources records of infected flies that have lived longer than the average time required by the strain they carry to complete its cycle in the fly and yet have no flagellates in their salivary glands.

PART I : *Section* I

ON THE LENGTH OF THE CYCLE

It may be said at once that it is rare for a strain of *T. gambiense*, freshly isolated from man in Uganda or its neighbourhood, to complete its cycle in *G. palpalis* in less than 20 days. In general, *T. rhodesiense* takes perhaps slightly less time to complete its cycle in this tsetse than does *T. gambiense*.

Kleine and Fischer (1913) compared the behaviour of 13 different strains of *T. gambiense* in *G. morsitans* and in *G. palpalis*, and found that the cycles in the former fly were slower, as is shown in the following Table. The length of the cycle was estimated by testing the flies during periods of 4 days on a series of clean monkeys. No gland dissections were carried out. As remarked above, flagellates may be found in the glands a few days before the fly is infective, and the accuracy of the estimate in these experiments of Kleine and Fischer depends on the infecting fly feeding promptly on the clean animal immediately it becomes infective.

Experiment No.	Length of developmental cycle	
	In *palpalis* (days)	In *morsitans* (days)
I.	26 to 30	41 to 44
II.	31 to 35	36 to 40
III.	...	...
IV.	...	40 to 47
V.	41 to 43	36 to 40
VI.	...	36 to 40
VII.	26 to 30	41 to 50
VIII.	...	...
IX.	22 to 25	31 to 35
X.	...	36 to 39
XI.	31 to 35	32 to 35
XII.	22 to 25	41 to 46
XIII.	23 to 25	41 to 43
Average	28 to 31	37 to 42

Bruce and his colleagues in Uganda, in their transmission experiments with laboratory-bred *G. palpalis*, found that ' the flies first became infective 28 days after the infective feed, and that after this time the flies are usually found to be capable of giving rise to the disease by their bites ' (Bruce *et al.*, 1911). They gave several instances where, in their opinion, the flies did not become infective until after the thirtieth day of the cycle. The earliest record of salivary gland invasion obtained by dissection was in a 25-day-old fly. The duration of the cycle was calculated by deducting a fixed incubation period from the time that elapsed before trypanosomes were first found in the test animal's blood.

The Luangwa Valley Commission, working in Northern Rhodesia with *T. rhodesiense* and *T. brucei* and *G. morsitans*, found that the cycle was completed in an average of 2 weeks : maximum 25, minimum 11 days (Kinghorn, Yorke and Lloyd, 1913).

Studying *T. rhodesiense* and *T. brucei* in *G. morsitans* in Nyasaland in 1912, Bruce and his colleagues, using the test animal method, concluded that for the *Human Strain* the minimum cycle was 14 days, the maximum 31, and the average 23 days ; for the *Zululand Strain* the minimum cycle was 13 days, the maximum 21, and the average 16 days ; and for the *Naturally Infected Dog Strain* (one satisfactory experiment), 24 days (Bruce *et al.*, 1915).

Lloyd (1930) in Nigeria, using dissection as his guide, found the glands invaded in an average time of 16 days (minimum 11—maximum 20) in experiments with strains of *T. brucei* and *G. tachinoides*.

At Mpumu, Fraser and Duke, and later the writer alone, carried out a number of transmission experiments with *T. gambiense* and *T. brucei*. The average cycle was about 30 days, though gland infections were found in flies of 25 days old. In contrast with these results, which depended on test feeds on clean animals and examination by fresh blood films, Robertson (1913), working at Mpumu at the same time and with the same material but using the much more accurate method of dissection, reported that : ' In one case a salivary gland was found infected on the twelfth day, but this is quite exceptional—on the sixteenth day infected glands are found in rare cases, and after the twentieth day they become increasingly frequent.'

Taylor (1932) examined 26 different strains isolated from man, using *G. tachinoides*. He found that ' Although the cycle of development in tsetse was identical in every strain tested, very well marked differences were observed in the time necessary for the completion of the cycle ' (p. 410). In two strains, Ayu V and Ayu IX, 25 per cent. and 20 per cent. respectively of the infected flies dissected on the thirteenth day of the cycle already showed gland infections. Dissection was seldom delayed beyond the twenty-first day, and by this time most of the strains tested had begun infection of the salivary glands (p. 410).

With one slow-developing strain, Gadau XIII, no gland infections were found before the nineteenth day, and only 18 per cent. of infected flies had gland infections by the twenty-first day, i.e., the date when the surviving flies were killed and dissected. Strain Gadau XV, tested in the cold season, ' did not commence invasion of the salivary glands until after the thirtieth day of infection, and in one batch of flies in which dissections were carried out from the twenty-first day onwards, the first salivary gland infection was seen on the thirty-eighth day ' (p. 410). Taylor observes that the behaviour of this strain may have been influenced by the cold season.

For strain Gadau XV the data given are as follows :—

Total dissected	281
' Gut-only ' infection	1
Gut and proventriculus	7
Gut and gland	1

These flies were dissected between the eighteenth and thirtieth days after their first infecting feed. The reference to a fly dissected on the thirty-eighth day thus suggests that more flies were examined than those reported. But, confining attention to the above figures, 7 flies out of 281 showed gut-and-proventriculus infections, and only 1 had flagellates in the glands. The ages of these 8 flies on dissection are not given, and it is not clear (to the writer) whether, after the twenty-first day, they were dissected as they died or whether they were chloroformed in groups at stated intervals.

His Table I shows that the flies used to transmit Strain Gadau XV infected a clean animal, but it is not stated if the date of this infection threw any more light on the duration of the cycle in this strain. The thirty-eighth-day-fly referred to is either the solitary gland-infected fly of the Table or one of others not included therein. If the former, why should not this fly have had its glands infected

TABLE I

Strain no.	Species	Infecting animal	Place of origin	Earliest evidence of completion of cycle in *G. palpalis*
I.	*T. gamb.*	M. 1st passage	E. P.	30th day gland
II.	„	goat 2nd passage	„	1st tested 31st day ; positive
III.	„	M. 1st passage	„	‘ Non-transmissible ’
IV.	„	Man	Buganda	1st tested 30th day ; positive
V.	„	M. 1st passage	Kavirondo, Kenya	24th day gland
VI.	„	M. 1st passage	E. P.	‘ Non-transmissible ’
VII.	„	Man	Buganda	1st tested 30th day ; positive
VIII.	„	„	„	‘ Non-transmissible ’
IX.	„	„	N. P.	28th day gland
X.		M. 1st passage	E. P.	29th day gland
XI.	„	„	„	18th day gland
XII.	„	Man	N. P.	23rd day gland
XIII.	„	„	„	21st day gland
XIV.		„	„	1st tested 24th day ; positive
XV.	„	„	„	24th day gland
XVI.	„	„	W. P., Lake Edward	24th day gland
XXVIII.	„	M. 1st passage	Bugungu	28th day gland
XXIX.	„	M. 1st, 2nd, 3rd passage	N. P.	‘ Non-transmissible ’, from European
XXX.	„	Man	Buganda	22nd day gland
XXXI.	„	„	N. P.	24th day gland
XXXII.	„	„	„	23rd day gland
XXXIII.	„	„	„	1st tested 26th day ; positive
XXXIV.	„	„	„	1st tested 25th day ; positive
XXXIX.	„	„	„	27th day gland
XXVII.	„	„	Buganda	28th day gland
XL.	*T. rhod.*	„	...	1st tested 28th day ; positive
XLIX.	„	M. 1st passage	Buzinza T.T.	1st tested 28th day ; positive
L.	„	„	„	1st tested 24th day ; positive
LI.	*T. gamb.*	M. 1st passage	E. P.	1st tested 25th day ; positive from European
LII.	„	„	E. P.	20th day gland

E. P. = Eastern Province
N. P. = Northern „
M. = Monkey
W.P. = Western Province
T.T = Tanganyika Territory

NOTE.—Save where the contrary is stated, no test was made on a clean animal.

for weeks before the day of its dissection ? Except for this strain, Gadau XV, all Taylor's strains had completed their cycle by, at the latest, the twenty-first day, a fact suggesting either that *G. tachinoides* is a readier transmitter of *T. gambiense* than is *G. palpalis*, or that the West Coast strains of this trypanosome are better adjusted to tsetse than are those of Eastern Africa.

Coming now to the Entebbe series of strains, these are presented in Table I. All strains of *T. gambiense* that have been transmitted at Entebbe are included, together with a few non-transmissible strains.

The Table shows that 15 of these strains, collected from all over the Protectorate, completed their cycle within 25 days. It will be noted that in the remainder, except for 4 described as non-transmissible, the flies were infective on the first day they were tested. None afford any evidence of requiring 30 days for completion of their cycle.

From the above resumé it is evident that, when the method of dissection is carefully applied, the biological cycle of *T. rhodesiense*, *T. brucei* and *T. gambiense* in tsetse is generally completed in 25 days. The Uganda strains show a remarkable constancy in this respect.

Kleine's experiments were conducted during the rainy season under what is described as a 'steppe' climate, hot days and cool nights, and 'different from the conditions prevailing in sleeping sickness.' With every strain save two the cycle took longer, sometimes much longer, in *G. morsitans* than in *G. palpalis*, a result which might be due to the two species reacting to the effects of temperature in different ways. In my own experiments with these two tsetse, no difference was discernible in the duration of the cycle. On the other hand, the *morsitans* pupae used in Kleine's experiment came from a fly belt not far removed from the scene of the experiment. It is noteworthy that in only one strain (V) was the cycle shorter in *G. morsitans* than in *G. palpalis*, and this strain took 10 days longer than any of the others to reach the glands in *G. palpalis*. Apart from this strain, the minimum average duration of the cycle in *G. palpalis*, expressed in terms not of gland invasion but of infectivity, as shown by Kleine's figures, is 25 days.

Kleine's method is accurate, provided that the flies feed often enough and the period on each successive test animal is short ; but it is not so accurate as dissection. His experiments are the most reliable evidence available of a cycle taking more than 40 days for completion at laboratory temperatures.

PART II

We will now consider trypanosome infections (*T. gambiense* and *T. rhodesiense*) in tsetse flies (*G. palpalis* and *G. morsitans*) that have lived 30 days or longer after their first meal on infected blood, these infections being restricted to the alimentary canal of the fly and not involving the salivary glands.

The array of figures is formidable, but the information they convey has a definite bearing on the whole question of the transmissibility of trypanosomes by Glossina. The Nigerian workers have reached conclusions different in certain essentials from my own, but the conditions in the two countries are so different that it is quite possible that both of us are right. My own views were partly influenced by the evidence now to be set forth. In Taylor's paper, the exact age of the flies on dissection is not stated, and so it is not possible to determine how many of the infected flies dissected at the end of each test had no flagellates in their glands.

An important point of divergence is this : Taylor believes that, once a fly is infected in the proventriculus, it is merely a matter of time before the glands of that fly also become invaded ; whereas I hold that in all but exceptionally transmissible strains there will always be a certain number of infected flies in which, no matter how long they live, the glands never will become infected. It is to support this contention that the figures of Table II have been assembled. I do not wish to impugn the accuracy of Taylor's conclusions, but merely to submit that he has not yet produced adequate evidence in their support. The existence in nature of non-transmissible strains is another point of difference between us, and it may well be that in Western Africa, the ancient home of human trypanosomiasis, the adjustment between the parasites and their hosts is more perfect than on the other side of the Continent It is probable, too, that *G. tachinoides* is a better transmitter of trypanosomes than *G. palpalis*, and it may be, as Taylor says, that all his strains are indeed transmissible by the local tsetse. But he has yet to prove that every fly with an infected proventriculus will, if it lives long enough, eventually develop an infection of its glands. In the paper under review no such proof is given. In view of the importance of the subject it is to be hoped that Taylor will be able to devote further study to the six strains in his Table which, according to existing standards are, *for all he shows to the contrary*, non-transmissible, i.e., those in which no gland infections were detected. None of the six was even included in his incubator series. Of these six perhaps the most important is Gadau VII, which produced no infections in 108 flies used in its examination. The point is surely of greater biological importance than the effect of temperature.

The subject before us at the moment is the individual fly, infected with a strain of *T. gambiense* known to be transmissible, that after living 30, 40 or even 50 days dies and is found to have no flagellates in its glands. The whole question of the liability of *T. gambiense* to lose the power of passage through tsetse is admittedly important, and infections of this kind bear on this problem. As Taylor remarks, if my conclusions are correct it would seem that *T. gambiense* strains are constantly becoming extinct in certain localities.

There is good reason to believe that this does occur, at all events in Eastern Africa. It is by no means uncommon for a careful inspection to reveal isolated cases of sleeping sickness living in a community where contact with fly is still maintained, and yet the disease shows no signs of spreading. Non-transmissibility of the trypanosomes in such an individual affords an excellent explanation of this phenomenon.

In the following Table are assembled all the data on this subject that I can discover, not only in the Entebbe records but in the reports of investigators in other parts of Africa. In the 'Age of Fly ' column the figures show the day after the first infecting feed on which the fly was dissected. In the great majority of the experiments the flies received two infecting feeds, separated by 48 hours' starvation.

In the column headed ' Earliest Evidence of Infectivity ' is shown the date after the first infecting feed upon which the flies were first tested on a clean animal. These tests were performed mainly in order to transmit the strain and not specially to test the duration of the cycle, and in consequence they supply but little information about the earliest date on which the flies became infective.

Table II shows that a fly may have its alimentary tract infected with a trypanosome during 5 to 7 weeks of laboratory service without becoming infective. It may be objected that the period of observation is inadequate. W. F. Fiske, lately Reclamation Officer, Uganda, an able student of tsetse, held that the average duration of life of a wild tsetse on Lake Victoria was probably from 2 to 2½ months, so

Table II

Species of trypanosomes	Series no. of strain	Infecting animal	Age, in days, of fly on dissection		Earliest evidence of infectivity of flies acquired by feeding them on clean animals	Remarks
			Earliest gland infection	'Gut-only' infections		
T. gambiense	II.	goat 340	42	40, 42, 42, 44, 44, 44, 44, 49, 49	1st tested 31st day ; positive	
	II.	M. 53	36	34, 35, 36, 36	not tested on clean animal	
	II.	sh. 332	none found	33, 33, 33, 49, 53, 56	flies not infective	
T. gambiense	III.	M. 113		36, 36, 36, 36, 37, 38, 39, 39, 42, 43, 44, 45, 46, 46, 46, 46, 46, 51, 51, 52, 53	674 flies were fed on animals infected with Str. III : the strain was never cyclically transmitted	
,,	III.	sh. 336		36, 36, 37, 37, 37, 37, 38, 38, 38, 38, 41, 41, 42, 43, 43, 43, 46, 50		
,,	IV.	man	33	34, 42, 46	1st tested 30th day ; positive	
,,	V.	M. 64	25	36, 36, 40, 40, 40		When, 5 months earlier, M. 64 was first exposed to clean flies, every infected fly that lived 24 days showed a heavy gland infection
,,	V.	sh. 161	24	31, 31, 31, 35, 39, 39		
,,	VI.	M. 65	none found	47		Strain was never cyclically transmitted
,,	VI.	goat 341	none found	34, 34, 42, 46		,, ,,
,,	VIII.	man	,,	50, 50		,, ,,
,,	VIII.	M. 120	,,	35, 37, 37, 37		,, ,,
,,	IX.	man	34	48	1st tested 30th day ; positive	Of 14 flies that lived 25 days or longer, 11 had gland infections
,,	IX.	goat 171	28	31, 31, 33, 34		

M = Monkey sh = Sheep

TABLE II.—*Continued*

Species of trypanosomes	Series no. of strain	Infecting animal	Age, in days, of fly on dissection		Earliest evidence of infectivity of flies acquired by feeding them on clean animals	Remarks
			Earliest gland infection	'Gut-only' infections		
T. gambiense	X.	M. 149	45	29, 40	1st tested 29th day ; positive	
,,	X.	goat 338	33	31, 31, 38, 38, 38		
,,	XI.	M. 162	18	28, 28, 28, 35, 37		
,,	XVI.	man	24	30, 35		
T. rhodesiense	XX.	various	25	31, 31, 31, 32, 32, 34, 34, 36, 40, 42, 42, 42, 50, 50		
T. gambiense	XXIX.	M. 506	none found	27, 28, 28, 29, 29, 30		Strain was never cyclically transmitted
,,	XXIX.	M. 507	,,	28, 29, 29, 29, 29, 30, 30, 30, 30, 30, 30, 30, 30, 30, 30, 30, 32, 32, 32, 32		,, ,,
,,	XXIX.	M. 601	,,	31, 31, 34		,, ,,
,,	XXIX.	M. 607	,,	29, 30, 30		,, ,,
,,	XXIX.	M. 547	,,	29, 29, 30, 30, 30, 30, 30, 31		,, ,,
T. rhodesiense	XLIX.	M. 972	20 (22, etc., etc.)	26, 26, 31, 32, 32, 34, 34, 36		
T. brucei	LIII.	M. 948	32	35		
,,	LIII.	M. 915	22	34, 34, 34		
,	LIII.	M. 960	30	30, 31, 31, 31, 31, 31, 31	1st tested 31st day ; positive	
Damba 1932	LVI.	M. 912	31	31, 32, 34, 34, 34	,, 26th ,,	
T. brucei	LVII.	M. 973	50	34, 50, 52	not tested till 45th day ; positive	

M = Monkey

TABLE II.—*Continued*

Species of trypanosomes	Series no. of strain	Infecting animal	Age, in days, of fly on dissection		Earliest evidence of infectivity of flies acquired by feeding them on clean animals	Remarks
			Earliest gland infection	' Gut-only ' infections		
T. rhodesiense	T.I.	M. 1015	29	31, 32, 33, 34, 34	1st tested 27th day ; positive	
,,	T.I.	M. 1040	25	30, 31, 31, 31, 32		
,,	T.I.	M. 1060	20	31, 32, 34, 34		
,,	T.I.	M. 1062	29	47	1st tested 22nd day ; positive	
,,	T.I.	M. 975	36	32, 36, 39, 42	,, 28th ,, ,,	
,,	T.I.	M. 1089	40	33, 35, 37, 43, 47	, 29th ,, .	
,,	T.I.	M. 1105	21	28, 35, 38, 43, 46, 48		
,,		M. 603	22	30, 30, 34, 36, 36, 36		
,,		g.-pig XXXIIC	16 (20, 22, 23, 23, 23, 23, 24, 25, 25, 27, 27)	27		These three strains being readily transmissible and highly infective to *G. palpalis*, fuller information is given about flies with gland infections, by way of emphasis
,,		M. 789	20 (23, 23, 26, 26, 26, 26, 26, 27, 27)	26, 26, 26, 26, 27, 27, 27		
,,		g.-pig. XIXa	25 (25, 25, 25, 25, 26, 26, 26, 26, 26, 26, 26, 27, 27, 27, 27, 27, 28, 28, 28, etc.)	25, 25, 25, 25, 25, 26, 26, 26, 26, 26, 26, 26, 27, 27, 27, 27, 27, 28, 28, 28, 29, 31, 31		
1924 Damba		M. 147	34	34, 35, 35, 35, 35, 35, 37, 37, 37, 37, 37, 38, 39, 48	1st tested 31st day ; positive	
,,		M. 178	34	30, 36, 37, 37, 37, 37, 37, 39	,, 30th ,, ,,	
,,		M. 180	36		,, 31st ,, ,,	

M = Monkey g.-pig. = Guinea-pig.

TABLE II.—*Continued*

Species of trypanosomes	Series no. of strain	Infecting animal	Age, in days, of fly on dissection		Earliest evidence of infectivity of flies acquired by feeding them on clean animals	Remarks
			Earliest gland infection	'Gut-only' infections		
1921 Damba		M. 171 M. 173 M. 227	27	29, 34, 36, 36, 51		
T. gambiense	K.I.			41, 41, 41	Infective 26th to 30th day (*G. palpalis*)	This group of experiments was performed by Kleine and Fischer (1913), cf. text page 454
„	K.I.			53, 53, 53, 53, 53, 53	Infective 41st to 44th day (*G. morsitans*)	
„	K.II.			35	Infective 31st to 35th day (*G. palpalis*)	
„	KIII.			35	none	
„	KIV.			58, 58	Infective 40th to 47th day (*G. morsitans*)	
„	K.V.			52, 52, 52, 52	Infective 36th to 40th day (*G. morsitans*)	
„	K.IX.			46, 46, 46, 46	Infective 31st to 35th day (*G. morsitans*)	
„	K.X.			45, 46, 46, 46	Infective 36th to 39th day (*G. morsitans*)	
„	K.XI.			46	Infective 31st to 35th day (*G. palpalis*)	
„	K.XI.			36, 46, 46, 46	Infective 32nd to 35th day (*G. morsitans*)	
T. gambiense			25	30, 31, 35, 36, 37, 40, 40, 49, 51, 53, 53		Bruce *et al.*, Uganda (1911) (*G. palpalis*)
T. rhodesiense			35	33, 34, 37, 43, 47, 50, 57	Infective 14th day	Bruce *et al.*, Nyasaland (1915) (*G. morsitans*)
T. brucei			31	30, 30, 31, 31, 33, 34, 34, 34, 34, 34, 34, 34, 35	„ 13th „	Bruce *et al.*, Nyasaland (1915) (*G. morsitans*)
„			50	42, 51	„ 24th „	Bruce *et al.*, Nyasaland (1915) (*G. morsitans*)

M = Monkey

that a trypanosome which cannot complete its cycle in less than 35 to 50 days will not stand much chance in nature.

Moreover, the evidence in Table II is not by any means the only evidence. There is the published work on non-transmissible strains in Uganda; there is an accumulation of observations awaiting publication on the behaviour of old laboratory strains in *G. palpalis* (and *G. morsitans*); and there is also the experience of Kleine and of Reichenow based on first-hand knowledge acquired in different parts of Africa. Taylor's figures show that in Nigeria *T. gambiense* completes its cycle sooner in *G. tachinoides* than *T. gambiense* does in *G. palpalis* in Uganda. They also show that some of his strains are only feebly transmissible by *G. tachinoides.* But they convey yet another suggestion, at variance with the author's own conclusions, namely, that there are strains in Nigeria which are non-transmissible by the local tsetse.

The discovery and recognition of non-transmissible strains of *T. gambiense* is at present largely a matter of chance, but a careful selection of cases will increase the likelihood of success. Patients recently infected are not at all likely to yield non-transmissible strains; cases of long standing, especially those which are progressing slowly towards a fatal termination, are the most promising. A mild infection does not necessarily signal a non-transmissible strain. On purely theoretical grounds, one would expect the Eket strains of Macfie and those of the Ayu district of Taylor to be readily transmissible, at all events in the earlier stages of infection; for in both these areas it would appear that the adjustment of the trypanosome to man, its principal or only mammalian host, approaches the high standard attained by *T. brucei* in the game.

But at the present stage of our knowledge it is difficult to foretell where non-transmissible strains will be found. Hitherto no such strain of *T. rhodesiense* has been recognised and only one of *T. brucei* (Strain XXIII of the Final Report of the League of Nations Commission). Probably this is due to the action of natural selection where vertebrate hosts are plentiful and in close contact with Glossinae. Evidently the phenomenon is most commonly associated with *T. gambiense* in nature.

The latest addition to the list of Uganda strains of *T. gambiense* that were non-transmissible by *G. palpalis* on their first isolation at the laboratory, came a few weeks ago from a man living on the shore of Lake Victoria, whose history strongly suggested that he had been infected for considerably more than 12 months. His symptoms were not serious and he was recognised as a case of trypanosomiasis during a special inspection of a labour gang. Up to date, some 600 flies have been fed upon monkeys infected from the patient by blood inoculation, and, although the boxes have all been placed in the incubator during the period of infecting feeding, no infected fly has yet been found. This strain is still under examination.

There remains nowadays no reasonable doubt that in Central and Eastern Africa there exist strains of *T. gambiense* which, on their first isolation from man, are incapable of completing their biological cycle in *G. palpalis*. Non-transmissible strains differ in their infectivity to fly; some produce numbers of 'gut-only' infections, others very few, others, again, none at all. Loss of transmissibility is obviously a serious matter for a trypanosome in nature, and it may be that an early manifestation of the retrogressive process is a lengthening of the time taken to complete the cycle. This is, however, at present mere speculation, and it is not uncommon to find strains of very low transmissibility completing their cycle in average time.

The evidence that has been reviewed in this paper shows that the great majority of strains of *T. gambiense* can complete their cycle in 25 days, and that many do so more rapidly. The formula for the transmissibility index given in the Final Report of the League of Nations Commission affords a means of comparing the transmissibility of different strains. With strains known to mature before the twenty-fifth day the earlier date is taken in completing the formula. In the absence of such information it would perhaps be more satisfactory to adopt the thirtieth instead of the twenty-fifth day as the date by which invasion of the salivary glands will be completed in all flies in which it is destined to take place. Then, provided 4-500 flies are used in a test, a useful conception will be obtained of the efficiency of a strain in its natural environment.

The evidence given and discussed in this paper suggests two conclusions :—

(1) It is very exceptional for the biological cycle of man's trypanosomes in Glossina to take more than 30 days for its completion. As a general rule, 25 days or less are required.

(2) With the majority of East African strains of *T. gambiense*

there will always be a certain number of individual infected flies in which trypanosomes never reach the salivary glands, no matter how long the flies live after their infecting feeding.

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A SIMPLE METHOD OF OBTAINING EGGS OF MOSQUITOES

BY

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When it is desired to study the eggs of a particular species of anopheline or aedine mosquito, it is usual to place a gravid female of the species in a small cage containing raisins and a dish of water, and to wait until some eggs are laid. This method often gives a disappointing result, even when the ovaries of the insect are ripe: either the insect dies before laying eggs or many days elapse before it does so. I have found that the following is a useful alternative plan:—

Transfer a fully gravid mosquito to a strong test-tube plugged with cotton wool, and shake the tube until the mosquito rests at the lower end. Then, holding the top of the tube in the right hand, tap the bottom end sharply seven or eight times against the lower part of the palm of the left hand (the 'heel of the hand'). Then drop the stunned mosquito into a watch-glass of water and cover it with a piece of glass. Within a few moments it will partially recover from the stunning effect, and oviposition will commence, an egg being laid every ten or fifteen seconds until 200 or more are lying on the surface of the water. By this method I have obtained eggs of the following English species:—*Anopheles maculipennis*, *bifurcatus*, *plumbeus* and *algeriensis*; *Aedes punctor*, *detritus*, *rusticus*, *maculatus*, *lutescens* and *caspius*; *Finlaya geniculata*; *Taeniorhynchus richardii*. P. J. Barraud, working in India, reports that by using the method he has obtained eggs of *A. pulcherrimus* which does not oviposit readily in the laboratory. The same plan, but without the preliminary procedure of stunning the mosquito, has enabled us to obtain eggs of several species which arrived at Horton by air-mail from abroad in Barraud's cages. It usually happens that some of the gravid female insects transported in these cages have their wings so damaged that they cannot fly, in which case all that is necessary is to transfer them to a watch-glass of water. In this way I have obtained eggs of *A. gambiae* (*costalis*) and *A. funestus* from Africa, *A. culicifacies* from India, *A. tarsimaculatus* and *albitarsis* from Trinidad, *A. maculipennis* from

Roumania and *A. hyrcanus* from Italy. At Horton the method originated from observations made in 1927, when it was decided that mosquitoes used in the procedure of infecting patients with malaria by inoculating sporozoites intravenously must be killed by stunning instead of by chloroform or ether.

If it is desired to rear anopheles from the egg-stage, some of the eggs should be transferred to one of the 'floating egg-harbours' invented and described by the late Dr. Malcolm MacGregor. In preparing the cork rings for these 'harbours,' I use dental wax instead of a softer variety.

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*TWO FURTHER CASES OF HUMAN INFESTATION WITH *BERTIELLA STUDERI* (BLANCHARD, 1891) STILES AND HASSALL, 1902, WITH SOME OBSERVATIONS ON THE PROBABLE SYNONYMY OF THE SPECIMENS PREVIOUSLY RECORDED FROM MAN

BY

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The adult forms of the cestode parasites of the genus *Bertiella* are normally parasitic in the intestines mainly of primates. Seven human cases have already been recorded. The first of these (Blanchard, 1913) was from Mauritius, where a creole child of eight and a half years of age passed fragments of a tape-worm, including the head, which were sent to Professor Blanchard and identified by him as *Bertiella satyri*. Some further segments of that same worm are still preserved in this laboratory. A second case was reported from India (Chandler, 1925) as occurring in Bengal in a Hindu child of two years of age ; in this case, both fragments and a complete worm were passed after treatment ; Chandler identified these specimens as belonging to the above species, *B. satyri*, and at the same time recorded its occurrence in a gibbon, *Hylobates hoolock*, in the Calcutta Zoological Gardens. The third case (*B. satyri*) was recorded from India (Mukerji, 1927) as occurring in a Hindu subject. The fourth case was from Cuba (Cram, 1928), but the patient, an adult Spaniard, had, eight years before, lived for a time in the Canary Islands, where infection may have occurred ; this worm was apparently a complete specimen with head, and was determined as a related species, *B. mucronata*. The fifth record was from St. Kitts (Cameron, 1929) ; the sixth (*B. studeri*) again from India (Maplestone, 1930) ; and the seventh (*B. studeri*) from Sumatra (Joyeux and Dollfus, 1931). The two further cases now recorded are again native children in Mauritius, one a local creole child, and the other of pure Indian descent but born in this country.

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*From the Bacteriological Laboratory, Medical and Health Department, Mauritius.

Case I.—A cestode parasite in a fragmentary state was sent to the laboratory by a local doctor, Dr. Duvivier, in 1929. It had been obtained from a creole child of about eight years old, after treatment with extract of male fern. The total length of the segments sent was about 70 cms. and no head could be found with the strobila. The fragments contained immature, mature and gravid segments which were all somewhat contracted, and thus the length of the portions received, in a relaxed state, would have been probably considerably greater than that recorded above. The gross morphology of the worm immediately called to mind the previous record of *Bertiella* in this country, but beyond a somewhat cursory examination no further notice of the specimen was taken until this year.

On recent full examination it was found that the somewhat fleshy segments are all broad and relatively short, the immature segments measuring about 6 mm. broad and 0·65 mm. long, whilst the gravid segments are about 15 mm. by 2 mm. Fragments stained with acetic acid-alum-carmine showed that the genital pores are irregularly alternate ; the testes are numerous (about 100) and form a continuous field between the longitudinal excretory canals, and lie along the anterior border of the segment and on its dorsal aspect ; the ovary is a large kidney-shaped organ lying in the poral half of the segment ; it occupies almost the total length of the segment and the inner portion extends towards, but never beyond, the medial line of the strobila ; the vitelline gland is definitely bilobed and situated just behind the centre of the large ovary and in its ' hilum,' while the shell gland lies about the centre of the vitelline gland. The cirrus pouch is rather difficult to delineate : it appears to arise just internal to the excretory canals and is a broad, almost globular, structure, with an internal seminal vesicle ; the vagina is fairly well developed, a single large receptaculum seminis is present, and immediately below this there is a well-marked vaginal dilatation. Some deeper staining cells in the walls, which are here apparently thicker, can be seen in the poral portion of the vagina. The genital ducts pass dorsal to the longitudinal canals. The uterus is at first a transverse sac lying between the excretory canals on either side ; later there are well-defined anterior- and posterior-branched sacculations arising from it, and in these posteriorly are seen ovigerous masses, the gravid segments eventually consisting virtually of a mass of eggs. Eggs were removed from a gravid segment before fixation, when the specimen was first brought to the laboratory, and were found to possess a rudimentary pyriform apparatus ; the average dimensions of a number of onchospheres were about 17·5 by 16·8 μ and of the outer capsules 50 by 45 μ. There appears no doubt that the specimen is one of *Bertiella.*

Case II.—While engaged recently in some other investigations at Beau-Bassin prison, some specimens of tapeworm were observed in a cabinet. By courtesy of Dr. Maingard, the prison medical officer, we were permitted to remove these for further study at the laboratory. The worm now recorded had attracted our attention particularly and was found to have been preserved in weak formalin in a bottle labelled as follows :—

> Passed by Warder Jagatsing's child girl of 4½ years—on 11.5.28 and 22.10.28. No head. Without medicine.

On examination the specimen was found to consist of three longish fragments, measuring in all about 80 cms., and a number of groups of segments. The specimen was in a very contracted condition, and a few groups of gravid segments appeared to have been left unfixed for some little time, as they had started to disintegrate ; the remainder of the worm was well preserved. One fragment of 23 cms. appeared to have been derived from immediately behind the head of the worm, but no head could be found. The gross morphology is that of the specimen already described, and, on staining portions the anatomy also conforms in every respect to that detailed above, except that the cirrus pouches are much better defined, narrower and more muscular. They arise just internal to the excretory canals. The segments immediately behind the head contain definite rudimentary genitalia, and by about the eighth segment the finer details of the testes, ovary and vitelline gland, together with their ducts, can be distinguished. The eggs dissected from a fixed gravid segment contain a rudimentary pyriform apparatus, but owing to the fixation are somewhat distorted. We have no reason to believe that the parasite is not the same species as that recovered from Case I. Examination of a number of specimens of stool for eggs or segments from this child in the last few weeks have failed to reveal any present infestation with the parasite.

On referring to the literature on the subject of the species of *Bertiella*, we have found that Blanchard (1913) lists no less than 18 species, but gives no key to their differential characters. Chandler (1925) gives a key to six of the species, the differential characters resting on the position of the head suckers, the length and relative size of the neck, if present, to the head, and also on certain differences of the genitalia. He states that in the case of *B. polyorchis*, *B. mucronata*, *B. conferta* and *B. cercopitheci* there is no pyriform apparatus in the egg. That this is not the case seems to have been demonstrated by Cram (1928). According to Cram, *B. mucronata* differs from *B. studeri* in that the cirrus pouch is atypical and very weakly developed, the vagina being strongly developed. Cram regards *B. satyri* as differing from *B. studeri* on somewhat similar grounds; she states that in *B. satyri* the cirrus pouch is feebly developed and the vagina is feebly developed in the outer half and has two enlargements, or receptacula seminis, in the inner half of its length; whereas *B. studeri* has a typical cirrus pouch strongly muscular in its development and the vagina is weakly developed. Baer (1927) reduces the recorded species of *Bertiella* to two, namely, *B. studeri* of the old world and *B. macronata* of the new. Meggitt (1927) describes a further species, *B. fallax*, in *Cebus capuchinus* from Egypt, and refers to *B. mucronata* (Meyner, 1895) as having been recovered from *Cercopithecus* sp. and *Mycetes niger*. He considers the differences between *B. cercopitheci*, *B. mucronata*, *B. conferta*, *B. polyorchis* and *B. studeri* to be non-specific and such as might be produced by variations in methods of fixation or in the degree of muscular contraction of the specimens; he considers that all these are but varieties of a single species, while *B. fallax* presents undoubted specific variation. Southwell (1930) also regards *B. satyri*, *B. cercopitheci* and a number of other species, among which *B. mucronata* is not listed as it probably has not been identified in India, as synonyms of the type-species *B. studeri*.

B. polyorchis (Linstow, 1905) was recovered from *Macacus cynomolgus* from Java. *M. cynomolgus* is the monkey occurring in large numbers in a wild state in Mauritius, and was originally introduced to this island from Ceylon. A large number of these animals are kept in captivity as pets, but we are unable to trace any relationship between such pets and these two further cases of human infection. The helminths of these local monkeys, as far as we are aware, have never been studied, so we are unable to state whether *Bertiella* occurs in them. In view of the slight and rather indeterminate differences between the majority of recorded species, and in spite of the fact that the two specimens described above present certain apparent differences in the structures of the cirrus pouches, it is, in our opinion, most unlikely that they belong to different species. A noteworthy point in our two specimens is the markedly bilobed form of the vitelline glands, a feature not apparently stressed in descriptions of the recorded species, but one which appears to us of more importance than a number of the minor differences in the structures of the genitalia which have received considerably more attention. We therefore endorse Meggitt's and Southwell's views on the synonymy of many of the recorded species, more especially of ***B. satyri***, ***B. mucronata*** and ***B. polyorchis***, with the type-species ***B. studeri***; and accordingly identify the two parasites herein described as ***B. studeri*** (Blanchard, 1891) Stiles and Hassall, 1902, and regard ***B. mucronata*** and other species mentioned above as synonyms. ***B. fallax*** alone would appear to present undoubted differential specific characters from the type-species.

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A NEW HAEMATOPHAGOUS SPECIES OF *MUSCA* FROM MALAYA

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Among a large collection of Muscid flies sent to me in 1932 by Dr. Green from Kuala Lumpur, I found a long series of both sexes of a new species of *Musca*, which I have much pleasure in naming after its discoverer. Later, Mr. Colbran J. Wainwright gave me a large collection of *Musca*, among which I found three females and many males of this Malayan species, which had been collected at Silchar, Cachar, Assam. I append a short description of the adults and of the male terminalia.

MUSCA GREENI sp. nov.

MALE. HEAD. Vertex linear, lower part and cheeks silvery ; antennae black, palps dark brown to black ; eyes with minute and sparsely distributed hairs (only visible under high magnification). MESONOTUM. Grey with four broad black stripes ; vein R_{4+5} with a row of small bristles on ventral side reaching almost to bend of M_{1+2}. ABDOMEN. Orange ; terga 1 and 2 orange with a median, rather narrow, black stripe, which expands anteriorly, forming a black patch ; tergum 3 orange with a narrow median black stripe expanding anteriorly T-like to form a band of varying width, a silvery patch, or even a stripe on each side of the median stripe, and the extreme margins silvery ; tergum 4 dark orange with a dark brown to black median stripe, a broad greyish-yellow stripe on each side, a greyish patch at each margin, and an admedian dark orange stripe ; tergum 5 very similar but all stripes and markings narrower. Sternum 1 dark brown to black ; sterna 2 to 5 inclusive orange ; about distal half of sternum 5 dark brown (fig. 1, a).

MALE TERMINALIA. Figs. 1, 2. ANAL CERCI. Free border only slightly emarginated, outer ends broadly rounded, inner produced into rather short but broad nipples. PHALLOSOME. Chitinous part long, about as long as membraneous part ; posterior process long, broadly expanded and deeply forked. PARAMERE. Anterior part

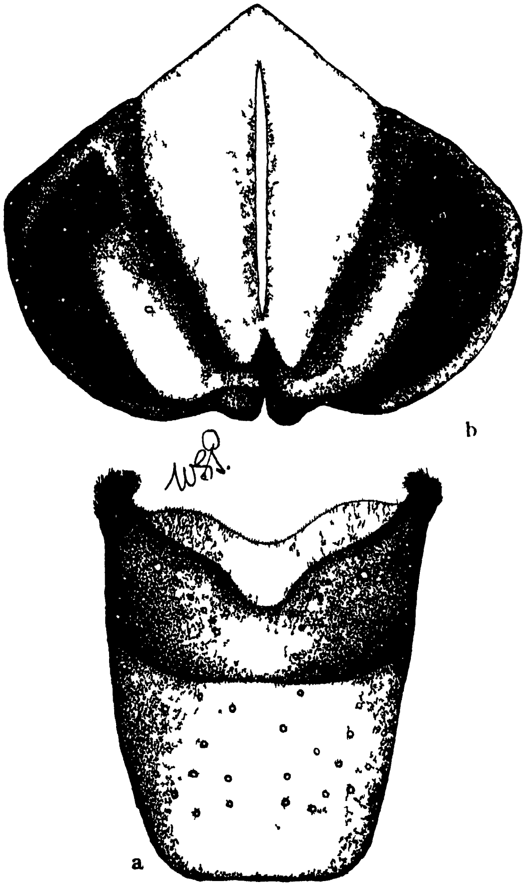

Fig 1. *a.*—Fifth sternum of *M greeni*, *b* —Anal cerci of same.

long and broad, and broadly rounded with well-marked posterior extension, and with two small and one long bristle ; posterior portion long, broad, well bent, and with the usual four to six sensory spines FIFTH STERNUM Long, longer than broad, about posterior half dark brown ; posterior processes short, broad, bent inwards and hairy.

FEMALE. HEAD. Vertex wide, about three-quarters width of an eye, lower part and cheeks grey ; vertical stripe about half width of vertex with straight edges ; outer vertical bristles in two rows ; eyes with a very few minute hairs (only visible under high magnification). MESONOTUM. As in male ; vein R_{4+5} with bristles as in male. ABDOMEN. Orange with extensive greyish-white markings ; terga 1

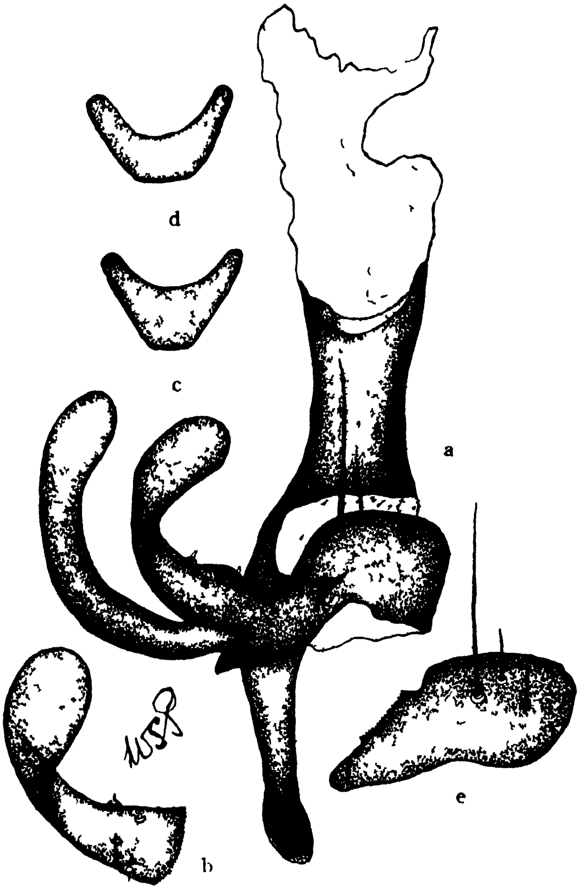

FIG. 2 *a*—Phallosome and one paramere of *M greeni* (Kuala Lumpur), *b*—Right posterior paramere of same (Silchar, Burma), *c*—Front view of posterior process of phallosome of same (Kuala Lumpur), *d*—Back view of posterior process of phallosome of same, *e*—Right anterior paramere of same (Silchar, Burma)

and 2 orange with a narrow median black stripe expanding anteriorly to form a large dark band, white patches at sides often forming greyish-white bands on each side of median stripe ; tergum 3 dark orange with extensive greyish-white markings, a narrow to broad dark brown median stripe, at low angle seen as a large brown median patch, a broad greyish-white stripe on each side, a large dark brown triangular patch with its base at the posterior border of tergum, and apex either reaching the anterior border or ending just short of it, and a lateral greyish stripe then appearing confluent with the greyish-white patches at sides of tergum ; tergum 4 very similar, but median stripe a broad triangular dark brown patch with apex towards posterior border; tergum 5 very similar but all stripes narrower.

EARLY STAGES. Unknown.

NOTES. *Musca greeni* is very like *M. bezzii* Patton and Cragg, *M. bakeri* Patton, and *M. illingworthi* Patton, the two latter occurring with it in Kuala Lumpur. In my paper on the Oriental species I shall point out how it can be distinguished from *bezzii*, with which it is closely related. The following 14 species are at present known to me from Malaya; I hope to give a key to their identification in the paper on the Oriental species.

GROUP 1

Musca vicina
„ *planiceps*

GROUP 2

Musca sorbens
„ *vetustissima*
„ *ventrosa*
„ *crassirostris*
„ *conducens*

GROUP 3

Musca xanthomelas
„ *craggi*
„ *illingworthi*
„ *bakeri*
„ *greeni*
„ *fletcheri*
„ *inferior*

MISCELLANEA

A MODIFICATION OF LLOYD AND JOHNSON'S METHOD OF PROBOSCIS AND GLAND DISSECTION IN TSETSE-FLIES

Instead of first removing the head with the glands from the thorax, the head and thorax preparation is laid on its back in normal saline, the left needle slightly pierces the thorax about between the third pair of legs to hold the specimen, while the right needle is laid horizontally across the base of the proboscis. By gentle pulling, the proboscis is pulled off the head and draws the glands out with it. It is easy to do and is successful with freshly killed flies.

J. F. CORSON.

23 *June*, 1933.

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OF
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This fellowship was founded in 1929 to commemorate the late Dr. Richard Caton, one of the original members of the governing body of the School, and for many years its Vice-Chairman.

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The following courses of instruction are given by the Liverpool School of Tropical Medicine each year :—

(1) Two courses for the Diploma in Tropical Medicine, commencing on the 3rd January, 1934, and the 1st October, 1934. The D.T.M. examinations are held in December, March and June.

(2) Two courses for the Diploma in Tropical Hygiene, commencing on the 11th January, 1934, and the 26th April, 1934. The D.T.H. examinations are held in March and June.

(3) Two courses in Veterinary Parasitology, commencing on the 3rd January, 1934, and the 1st October, 1934.

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OBSERVATIONS ON THE DEVELOPMENT OF *PLASMODIUM MALARIAE* LAV. IN *ANOPHELES ELUTUS* EDW.

EXPERIMENTAL TRANSMISSION OF QUARTAN MALARIA TO MAN FROM THE MOSQUITO

BY

G. MER

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(*Received for publication* 13 *September*, 1933)

The experiments reported below were carried out in the Huleh area, where quartan malaria predominates throughout the whole year. Local epidemiological observations on quartan malaria have shown that new infections occur from June to October, and our experiments were, therefore, carried out in summer (1932 and 1933).

Carriers selected among children of the Huleh were used for infecting mosquitoes. During summer, it is difficult to find carriers of *P. malariae* whose blood is entirely free from gametocytes of *P. falciparum*, but in the cases selected the latter were sufficiently scarce not to interfere with the experiment. Actually these precautions, though useful, were not essential, for, as will be shown later, there would be no difficulty in distinguishing mixed infections of these two parasites in the mosquito.

EXAMINATION OF THE CARRIER

Thick drops and thin films were examined 3-4 hours before the experiment and during the experiment. Some slides were stained by the routine method, others were kept in a moist chamber for 10, 20 or 30 minutes in order to study exflagellation.

Only laboratory-bred specimens of *A. elutus* were used. After the feed on a carrier, they were kept in a moist room and fed on raisins, except in a few instances indicated below. They were dissected at various intervals.

EXPERIMENT 28.

30.8.32. Room temperature 37° C.
Carrier A.N.A., aet. 3.
P. malariae. Asexual forms 2 to 40 leucocytes.
Gametocytes 1 to 150 leucocytes.
P. falciparum. Gametocytes 1 to 5,000 leucocytes.
Exflagellation observed in fresh preparation.

Anopheles hyrcanus : 15 fed on above carrier and dissected at intervals up to 15 days. All were negative.

A. elutus : 11 fed and dissected up to 21 days after the experimental feed. Two were found positive ; one, dissected 14.9.32, contained 4 oocysts in midgut ; one, dissected 19.9.32, contained 2 oocysts in midgut. In both cases the salivary glands were negative.

EXPERIMENT 49.

19.10.32. 10 a.m. Room temperature 24° C.
Carrier H.M.N.
P. malariae. Asexual forms 4 to 30 leucocytes.
Gametocytes 1 to 72 leucocytes.
Ratio ♀ : ♂ 1 : 2.
P. falciparum. 1 to 2,050 leucocytes.

A moist chamber preparation was examined and subsequently stained. A quarter of all the microgametocytes exflagellated.

Eighteen *A. elutus*, hatched on 17.10.32, fed on this carrier, and 7 were subsequently found to be infected ; 4, dissected after 13 days, contained oocysts in the midgut ; 1, after 30 days, contained a few sporozoites in the salivary glands ; and 2, after 47 days, contained numerous sporozoites in the salivary glands.

FEEDING EXPERIMENTS ON VOLUNTEERS.

10.11.32. Six mosquitoes, of which 3 were subsequently found positive, fed on a human being who had never had malaria.

Result negative during an observation period of 9 months.

This result was inevitable, for, as was subsequently found, sporozoites do not invade the salivary glands until about 28 days after the infecting feed.

23.11.32. Five mosquitoes fed on volunteer Z.M.

5.12.32. Two of the above mosquitoes were found to contain numerous sporozoites in their salivary glands.

Result. Z.M. acquired an infection with *P. malariae.* For details, see below.

EXPERIMENT 69.

6.6.33. Room temperature 25° C.
Carrier M.S.M.K., aet. 2.
P. malariae. Asexual forms 2 to 30 leucocytes.
Adult gametocytes : 1 to 120 leucocytes.
Ratio ♀ : ♂ 9 : 8.
P. falciparum. Gametocytes 1 to 5,000 leucocytes.
Spleen 3 fingers below costal margin.

Moist chamber preparation ; two-thirds of all the microgametocytes exflagellated within 30 minutes.

Twenty-six *A. elutus* fed on this carrier and were kept at a temperature of 19° C. to 26° C. between 6.6.33 and 11.7.33. Four mosquitoes were subsequently found infected.

3.7.33. In one mosquito 15 oocysts, 45μ to 60μ in diameter, were found. The three largest oocysts contained sporozoites, and a few sporozoites were found in the salivary glands.

7.7.33. Five mosquitoes placed on a malaria-free volunteer, B.Y. Two, which did not ingest blood, were dissected, and 1 was found to contain 2 unripe oocysts, 48μ in diameter, and numerous sporozoites in the salivary glands (obviously derived from one or more ripe oocysts which had burst).

The other three mosquitoes were dissected on 11.7.33 and two were found positive ; one contained 5 unripe oocysts, 32μ to 53μ in diameter, and the other contained 5 unripe oocysts, 32μ to 60μ in diameter. In both, the salivary glands were negative.

B.Y. subsequently became infected with *P. malariae*. For details, see below.

EXPERIMENT 74.

16.6.33. Room temperature 25·5° C.
Carrier A.S.A., aet. 9.
P. malariae. Asexual forms 1 to 7 leucocytes.
Young gametocytes 1 to 40 leucocytes.
Fully grown gametocytes 1 to 50 leucocytes.
Ratio ♀ : ♂ 2 : 3.
Leucocytes 6,000 per cm.
Spleen palpable.

Thirty-six *A. elutus* fed and were kept at 23° C. to 26° C. Of these, 8 were subsequently found positive.

27.6.33. Two mosquitoes contained 15 and 4, respectively, very transparent oocysts, 9μ to 18μ in diameter.

28.6.33 One mosquito contained 2 oocysts, 15μ in diameter. Nine *A. elutus* fed on a malaria-free subject. Of these, 4 were subsequently found positive.

5.7.33. One contained 1 oocyst, 32μ in diameter, and one contained 5 oocysts, 24μ to 33μ in diameter.

14.7.33. One contained a ripe oocyst which burst during the dissection and liberated a large number of sporozoites.

17.7.33. One contained an unripe oocyst, 47μ in diameter, and sporozoites in the salivary glands.

17.7.33. Two *A. elutus* fed on volunteer D.B., who had never had malaria. Of these, 1 was found positive on 20.7.33. One unripe oocyst, 39μ in diameter, was found in the midgut, and a few sporozoites were found in the salivary glands.

Volunteer D.B. was subsequently found to be infected with *P. malariae*. For details, see below.

RESULTS OF INFECTION EXPERIMENTS

All the volunteers were kept in Rosh Pinah, a village in which no cases of quartan malaria have been found during the past five years.

EXPERIMENT 49.

The volunteer Z.M., on whom 5 *A elutus* (of which 2 were subsequently found to have sporozoites in the salivary glands) fed on 23.11.32, had headache and general indisposition on 18.12.32 and again on 21.12.32. Temperature normal. Blood negative.

24.12.32. Headache. Temperature, 38·7° C. Rigor at 4 p.m. ; a few rings and sporulating bodies of *P. malariae* found.

27.12.32. Rigor at 4 p.m. Quartan rings and schizonts found in blood.

28.12.32. Treatment commenced ; 0·9 grms. quinine and 0·03 grms. plasmochin per day for 15 days. No relapse.

EXPERIMENT 69.

The volunteer B.Y., on whom 5 *A. elutus* (of which one was subsequently found to have sporozoites in the salivary glands) were placed on 7.7.33, had a temperature of 37·9° C. on 20.7.33.

20.7.33. Blood negative.

31.7.33. General indisposition. Blood negative.

2.8.33. 8 p.m. Temperature 38° C. Rigor. A few rings of *P. malariae* found.

3.8.33. 10 a.m. Temperature 39° C. A few rings of *P. malariae* found.
5 p.m. Temperature normal.

5.8.33. 8.30 p.m. Rigor. At 10 p.m., temperature 40·8° C. One ring to 500 white cells, and one rest of a sporulating body (containing three merozoites).

6.8.33. 1 a.m. Temperature 39·9° C. One ring to 300 white cells.
4 p.m. Temperature 37° C. One ring to 7,000 white cells. One young gametocyte found.
10 p.m. Two trophozoites (typical band forms) to 5,000 white cells found.

7.8.33. 4 a.m. Temperature 36·8° C. One trophozoite to 500 white cells.
10 a.m. Temperature 36·5° C. Idem. 4 p.m. Temperature 36·5° C. Idem.
10 p.m. Temperature 36·3° C. Three trophozoites and 1 microgametocyte to 2,500 white cells.

8.8.33. 4 a.m. Temperature 36·3° C. Commencement of division. Forms with 2 masses of chromatin found.
10 a.m. Temperature 36° C. Chromatin divided into 4 masses.
4 p.m. Temperature 36·9° C. Five sporulating bodies (6 merozoites in each), and 1 microgametocyte per 5,000 white cells.
8.30 p.m. Rigor. Temperature 38·2° C. One sporulating body with 6 merozoites to 5,000 white cells, and 1 ring to 100 white cells.
9 p.m. Temperature 39·9° C. Six sporulating bodies (1 with 12, the rest with 6 merozoites) to 5,000 white cells. One ring to 100 white cells. Two out of 65 rings show 2 masses of chromatin (fragmentation of nucleus).

9.8.33. 4 a.m. Temperature 37·3° C. One ring to 62 white cells. No sporulating bodies to 5,000 white cells.
7 a.m. One ring to 62 white cells. The largest rings contain pigment.

EXPERIMENT 74.

The volunteer D.B., on whom 2 *A. elutus* (of which one was subsequently found to have sporozoites in the salivary glands) had fed on 17.7.33, had headache and general indisposition on 11.8.33. Blood negative. On 13.8.33, at 4.30 p.m., rigor ; temperature 38·3° C. Blood negative.

14.8.33. 7 a.m. Temperature 37·8° C. Blood : a few rings of *P. malariae* found.

COMPARISON OF LIFE CYCLE OF *P. MALARIAE* TO THOSE OF *P. VIVAX* AND OF *P. FALCIPARUM*

The development of *P. malariae* in *A. elutus* is much slower than that of *P. vivax* and *P. falciparum*. Under the conditions of the experiment recorded above, the two latter parasites complete their development up to the presence of sporozoites in the salivary glands within 17-18 days. This was determined in parallel experiments on gametocyte carriers of *P. vivax* and *P. falciparum* carried out under the same conditions. After 12 days' development, oocysts of *P. vivax* are up to 30μ, those of *P. falciparum* up to 36μ in diameter, while those of *P. malariae* are only from 8 to 15μ in diameter. At this stage the oocysts of *P. malariae* can easily be overlooked, partly because of their small size and their transparency, and partly because they contain relatively less pigment than the oocysts of *P. vivax*, and, in addition, the pigment of the young oocysts of *P. malariae* is apt to be distributed at the periphery.

In *P. vivax*, ripe oocysts up to 40μ in diameter containing sporozoites were found after 17 days or earlier ; in *P. falciparum* (up to 54μ in diameter), after 18 days or earlier ; while in the case of *P. malariae* oocysts (up to 60μ in diameter) with active sporozoites were found after 28 days.

The salivary glands were not infected till 28 days after the infecting feed in the case of *P. malariae*, while in the other two species they were found to be infected within 17 and 18 days respectively. The sporozoites of *P. malariae* examined *in vivo* were found to be larger than those of the other two species ; their movements are more rapid and their extremities are curved. The comparative size of the sporozoites of the three species are *P. malariae* 11, *P. falciparum* 9, *P. vivax* 8.

In other experiments, 202 specimens of *A. elutus* were dissected 15 days and more after feeding on 15 patients containing gametocytes of both sexes in their blood, and were found negative. Eleven of these patients had no young gametocytes in their blood. Of these, only 6 did not show exflagellation of microgametocytes.

We do not as yet attempt to explain these negative results in spite of the exflagellation, and the experiments await further analysis ; but the fact that the best results were obtained with those carriers which contained both young and fully grown gametocytes suggests the possibility that old macrogametocytes are either not fertilized or are incapable of further development after being fertilized.

The relatively long period necessary for the development of *P. malariae* in *A. elutus* indicates that a large number of cases of quartan malaria can only occur under conditions which favour longevity in the adult mosquito. Such conditions obviously occur in the Huleh area, where there is a constant high relative humidity enabling these insects to survive for more than 30 days at a high temperature, and where the breeding places are in close proximity to the feeding grounds.

SUMMARY

A. elutus was infected with *P. malariae*.

The development of the oocysts is described and compared to that of *P. falciparum* and *P. vivax* in the same mosquito. The salivary glands are invaded after 28 days at a temperature of 23°-26° C.

The sporozoites of *P. malariae* are larger and more active than those of *P. falciparum* and *P. vivax*.

Three human beings were infected by the bite of infected *A. elutus*.

The development of *P. malariae* was studied in one experimental infection. Nuclear division in the schizonts does not occur until about 16-24 hours before the rigor.

CUTANEOUS ONCHOCERCIASIS IN A *SIMULIUM DAMNOSUM*-INFESTED REGION OF UGANDA*

BY

E. G. GIBBINS

AND

L. J. A. LOEWENTHAL

(*Received for publication* 2 *October*, 1933)

CONTENTS

PAGE

I. INTRODUCTION 489
II. GEOGRAPHICAL DISTRIBUTION 490
III. OBSERVATIONS IN UGANDA 491
IV. DISCUSSION 493
V. REFERENCES 496

I. INTRODUCTION

Infestation with *Onchocerca volvulus* Leuckart, 1893, in association with the presence of simuliid flies has been reported from various parts of tropical Africa. The earliest reference to the disease is that of O'Neill in 1875, quoted by Brumpt (1927), who found a microfilaria in the skin of six patients suffering from ' craw-craw ' Since this report, the condition known as ' tropical lichenification,' ' pseudo-ichthyosis,' ' la gale filarienne ' and (partly) ' craw-craw ' have been ascribed to the passage of the larvae of *O. volvulus* in the skin, notably by Montpellier and Lacroix (1920). Contrary to this view should be quoted the observations of Brumpt (1920), who had seen the skin condition in parts of tropical Africa where *O. volvulus* was then unknown, of Macfie and Corson (1922), and of Corson (1922), who wrote : ' No causal relationship between the larvae and the conditions of " craw-craw," elephantiasis and lichenification was shown. . . .' Nevertheless, it is now believed that the larvae of *O. volvulus* are responsible for these dermatoses, if not for many cases of elephantiasis (Ouzilleau, Laigret and Lefrou, 1921 ; Strong, 1930 ; and others).

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*We are indebted to the Director of Medical and Sanitary Services, Uganda, for permission to publish this paper ; to Dr. N. J. Willans, for histological assistance ; and to the administrative officers of the Buganda and Eastern Provinces, who greatly facilitated our investigations.

Although the disease has been known for over half a century, it is only in comparatively recent years that an insect has been suspected of being the vector. Attention was first directed to a simuliid fly by Dry (1922), who alluded to the possibility of a *Simulium*, subsequently determined as *S. neavei* Roub. (De Meillon, 1930), being concerned in the dissemination of a disease in Kenya which, from his description and photographs, was undoubtedly onchocerciasis. It was not, however, until several years later that Blacklock (1926*a* and 1926*b*) proved by dissection that *S. damnosum* Theo. was the carrier of *O. volvulus* in Sierra Leone. Confirmation of Blacklock's researches is found in the work of Bequaert (1930), in Liberia, who demonstrated living embryos in the gut and thorax of *S. damnosum*, and of Hissette (1932), in the Belgian Congo, who found the developmental stages in *S. damnosum* and *S. neavei*.

II. GEOGRAPHICAL DISTRIBUTION

Cutaneous onchocerciasis has been reported from the following countries :—

West Africa :—

French Sudan, Guinea and Ivory Coast, Laigret (1929).

French Guinea, Clapier (1917).

Senegal, Dahomey and French Niger, Montpellier and Lacroix (1920).

Liberia, Strong (1930).

Sierra Leone, Blacklock (1926).

Central Africa :—

Belgian Congo, Brumpt (1903), Ouzilleau *et al.* (1921), Dubois (1916 and 1917), Rodhain (1920), Strong (1930), Blanchard and Laigret (1924).

East Africa :—

As far back as 1899, Sir Albert Cook (quoted by Manson-Bahr, 1929) noted the presence of *Onchocerca* nodules in several natives in the Busoga district of Uganda, but in a personal communication he informs us that, as far as he can recollect, lichenification was not observed. Lichenification, however, has been reported from various parts of East Africa : Brumpt (1903) found it between the Nile and Lake Rudolf ; Dry (1922) pictures and describes briefly the condition in the Lumbwa Reserve in Kenya ; and

Dr. Kauntze has informed us that he has seen a typical case of lichenification in the same country. Fleming and Hennessey (1932) described five cases of tropical lichenification in natives of Uganda.

In view of our findings in cases of lichenification, it is of interest to note that Brumpt (1927) states that the microfilaria of *O. volvulus* had never been found in the skin, except in West Africa, though the skin disease had been reported from the East to the West Coast.

III. OBSERVATIONS IN UGANDA

During the past 18 months, one of us (L.J.A.L.) has seen 54 cases in the course of the routine work of the Skin Department of Mulago Hospital, Kampala. In the anamnesis, some of the patients blamed the ' mbwa ' fly* as causing their disease, and this led us to compare the distribution of the cases with that of *Simulium* and to find that they almost coincided. Indeed, many of the natives regard it as a disease, known as ' lukuku,' peculiar to the districts of Kyagwe and Busoga (see map) ; these regions are for the greater part heavily infested with *S. damnosum.*

Buwenda.— In a previous investigation, one of us (E.G.G., 1933) studied the bionomics of *S. damnosum* near the source of the Victoria Nile, and found a dense infestation with adults of this species in the village of Buwenda, near Jinja, Busoga. This was the site of our first enquiry in June, 1933, which coincided with a period of high density and activity of this fly. Practically all the inhabitants were found to be affected by lichenification in various stages. At this village, 112 wild *S. damnosum* were caught while biting the inhabitants, and of these 48 were dissected ; 7 showed embryos in the thoracic muscles, and one of these had also 2 active nematodes in the head. Twenty-one persons suffering from lichenification were examined, biopsies were obtained from 13 ; of these, 9 were found to be infected. Time did not permit of further investigation in this locality, though both patients and flies were numerous.

Busana and Nagoje.—The country lying to the west of the Nile, from which our earlier cases had come to Kampala, was next visited ; the districts selected were Busana, Bugerere, and Nagoje,

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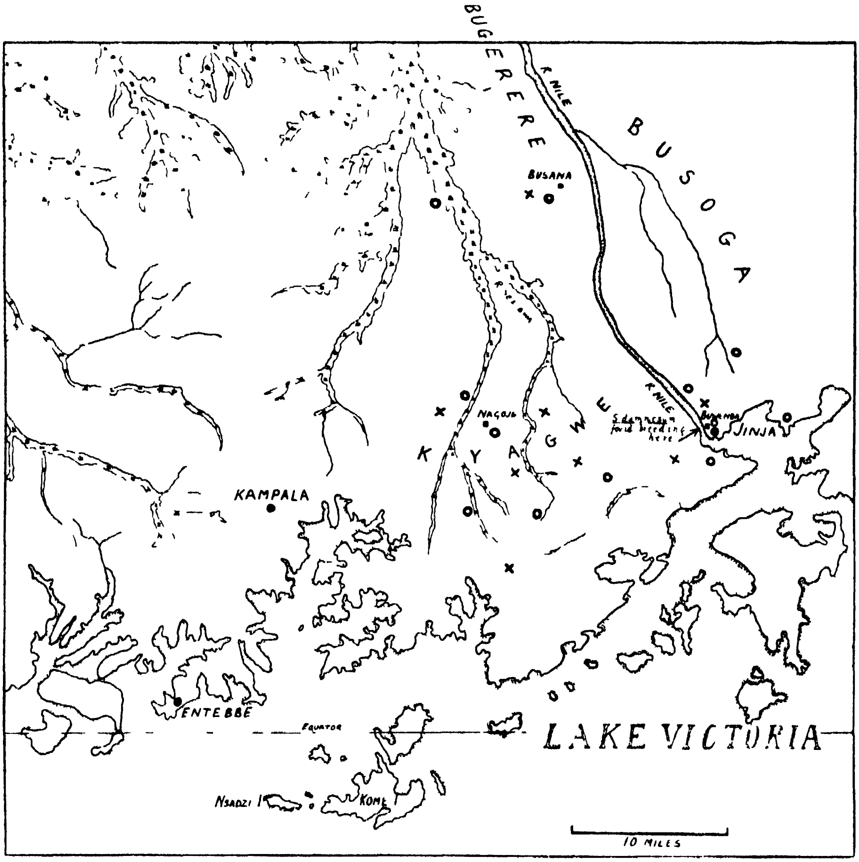
In the areas inhabited by these people, this insect was found to be *Simulium damnosum.

Kyagwe. Adults of *S. damnosum* were active at both places, but were more numerous at Busana. Of 33 captured off the inhabitants, 2 were found infected in the gut among 15 dissected at Busana; while at Nagoje, of 37 taken outside the village while feeding on two small boys apparently unaffected by the disease, among the 12 dissected none was found to be infected. The disease was more prevalent at Busana, the cases seen at Nagoje having been summoned by the chief from within a radius of four miles ; 4 biopsies were taken at Busana and 5 at Nagoje, while blood-counts were carried out on 4 other cases. For purposes of comparison, blood-slides were taken



Map of part of Uganda showing the site of our observations. The crosses (×) show the place of origin of cases of onchocerciasis either seen at Kampala or studied on the spot, and the circles (O) indicate the actual localities where *Simulium damnosum* was captured in the act of biting man.

from 5 prisoners in Nagoje ; these men showed no signs of the disease and no apparent *Simulium* bites.

Kome Island. –This island (population 108) situated in Lake Victoria, where *S. damnosum* is absent though *S. adersi* (E.G.G., 1934) is prevalent at certain seasons, was next visited for purposes of control. At the time of our visit, *S. adersi* was not active and no specimens were captured ; a dermatological survey of the population was made without discovering a single case of lichenification, and 30 biopsies, examined in saline by the method described by Blacklock and Southwell (1931), were negative.

TABLE I

Showing infection of *Simulium damnosum* with nematodes

Date	Village	Number captured	Number dissected	Infected			
				Gut	Thorax	Head	Head and thorax
26.6.33	Buwenda	112†	48	—	7*	1	1
2.7.33	Busana	33‡	15	2	—	—	—
3.7.33	Nagoje	37§	12	—	—	—	—

*The embryos resembled some of the stages described and figured by Blacklock (1926*a* and 1926*b*).

†Caught while biting man in an endemic area of onchocerciasis.

‡Caught while examining patients under an open shelter.

§Caught on two small boys (not apparently affected with onchocerciasis) near a stream some distance from the village.

IV. DISCUSSION

Clinical. The accurate description of Brumpt (1927) relating to cases of cutaneous onchocerciasis in West Africa leaves little to add. Our cases presented all stages of the disease, from papular and papulo-pustular to a generalised lichenification, in some individuals amounting to a true pachydermia. Once the true nature of the disease became apparent to us by the finding of the causative nematode (i.e., after the visit to Buwenda), a routine examination for subcutaneous nodules was undertaken ; such nodules are found in about one-third of our cases of lichenification, and their dissection reveals adult nematodes.

In certain advanced cases, possibly owing to the prolonged irritation, symptoms were noted comparable with those described

TABLE II

Examination of cases

Locality	Eosinophils per cent. in blood	Kahn test	Larvae in stained sections	Adult worm in sections
Buwenda	42	+	+	—
	50	+	+	—
	67	±	—	—
	13	+ ±	+	—
	36	+	+	—
	67	+ +	—	—
	26	—	—	—
	36	+	—	—
	17	+ +	+	—
	21	+	+	—
	67	±	+	+
	18	+	+	—
	37	+	+	—
Busana	32	Not done	+	—
	17		—	—
	31		+	—
	19		—	—
Nagoje	35	Not done	+	—
	71		—	—
	29		—	+
	29		+	—
	21		—	—
	45	Not done	... ...	Not done
	45			
	27			
	39			

Notes to Table II

(i). In the Nagoje cases, the average eosinophilia was 37·3 per cent.; highest 71 per cent., lowest 21 per cent. In the 5 prisoners whose blood was taken for comparison, the average eosinophilia was 11·4 per cent. (normal for the general population); highest 29 per cent., lowest 1 per cent.

(ii). Kahn tests were taken in the first series. As the results were comparable with those for the average native inhabitants, blood was not taken for the Kahn test in subsequent series.

(iii). The larvae seen in these sections, as well as others isolated later by the teasing method, were found to be morphologically indistinguishable from those of *Onchocerca volvulus* Leuckart.

by Montpellier and Lacroix (1920). No erysipeloid attacks have been noted, nor has our attention been drawn to ocular complications (Hissette, 1932).

It is by no means certain that all cases of lichenification are due to the microfilariae of *O. volvulus*. We have seen cases, clinically indistinguishable from cutaneous onchocerciasis, in whom the thickening of the skin had apparently followed other itching dermatoses, such as scabies. These cases had no subcutaneous nodules ; they had not resided in *S. damnosum*-infested areas, and larvae were not found in repeated biopsies. It is just possible that the skin changes in onchocerciasis are due to long-standing attacks by the *Simulium*, whose bite can irritate for a period of a week.

Out of 31 cases questioned, 29 had immigrated from *S. damnosum*-free areas some years before experiencing the disease. The question of tolerance to the larvae among the indigenous population has yet to be investigated ; it is noteworthy that many subjects presenting *Onchocerca* nodules have a skin intensely infested with larvae, but have neither symptoms nor signs of lichenification.

Entomological.—Of the numerous species of *Simulium* known to occur in Uganda, information on their blood-sucking habits is scanty. In the time at our disposal it was not possible to visit regions haunted by species other than *S. damnosum* and *S. adersi*. *Simulium neavei* occurs in the Ankole and Toro districts, but its relationship to onchocerciasis in the country remains to be investigated. It is highly probable that further investigations into the distribution of the disease will be the means of incriminating other species.

The few dissections of *S. damnosum* carried out are sufficient to indicate the danger of this small fly in an endemic area of the disease where as many as 14 per cent. were found infected. Control of the insect will be a problem for the near future ; huge tracts of extremely fertile country are rendered uninhabitable through the ravages of the fly. The lives of those who settle within the infested areas, owing to their inability to secure suitable land elsewhere, are made exceedingly miserable by the irritations of the bites from this tenacious pest. While cultivating, the natives cover themselves as much as their means allow them ; banana-leaves are wrapped round the legs, and those on Government duty are issued with pantaloons. It has been suggested in a previous paper by one of us (E.G.G., 1934) that trapping may be found to be a practicable method of reducing its prevalence and rendering these districts habitable ; a study of the breeding habits of this species (E.G.G., 1933) has proved the futility of attempting to attack the fly in its early stages.

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PRELIMINARY NOTE ON A FOCUS OF *S. MANSONI* INFECTION IN SIERRA LEONE

BY

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For several years, occasional cases of infection with *Schistosoma mansoni* have been recorded from various parts of Sierra Leone, but the source of these cases was not traceable and no focus of infection has yet been recorded.

During his investigation into the prevalence and transmission of human schistosomiasis in Sierra Leone, Blacklock (1924) visited Kabala in the Northern Province of the Protectorate in February, 1924, but he does not record the presence of cases nor of any snail there ; Connolly (1928), in ' The Non-Marine Mollusca of Sierra Leone,' has no record for Kabala. Blacklock and Thompson (1924) remark on the ' rarity of snails of the genus *Planorbis* and the absence of Intestinal Schistosomiasis.' Blacklock (1930), in his ' Report on a Survey of Human Diseases in the Protectorate of Sierra Leone,' says : ' It would be of great interest to know the source, or sources, of infection, of the occasional cases harbouring *S. mansoni* which we have encountered in Sierra Leone ; at present it may be said that this question is still unanswered ; there is, however, no evidence that infection with *S. mansoni* is either common or constitutes a problem here.'

During 1930, two cases of *S. mansoni* infection were discovered by the writer in Kabala ; in 1931, two other cases were found ; one of the 1930 cases and one of the 1931 cases were residents of Kabala. On the writer's return from leave in the early part of 1932, four more cases were seen within a few weeks, a fact which led to the suspicion that the disease was increasing.

Cases continued to occur, and during the year 1932 28 were seen, followed by 9 further cases in 1933 up to the beginning of June, when the writer left for leave ; the last case was seen at the end of March. Of these 37 cases, 33 were residents of Kabala, one had been living there for nine months, and two others were known to be visitors who had been living in Kabala for eight or nine weeks ;

one case was stated to have come from a town to the north. The case histories proved beyond doubt that the disease was being propagated in Kabala.

The age and sex distribution of the 1932 and 1933 cases are set out below.

Age	Male		Female	
	1932	1933	1932	1933
0-10 years ...	6	1	3	2
11-20 „ ...	1	—	2	2
21-30 „ ...	2	—	4	2
31-40 „ ...	2	—	4	2
41-50 „ ...	2	—	1	—
51-60 „ ...	1	—	—	—
Totals	14	1	14	8

On April 5th, 1932, the Kabala stream was visited and large numbers of a species of *Lymnaea* and also of a species of *Planorbis* were discovered. Later on in the investigation a few specimens of a species of *Bulinus* were collected. Snail dissections were carried out, and those of the *Planorbis* species were found to contain cercariae of a morphology similar to that of human schistosome cercariae.

The banks and bottom of the stream were cleaned for a considerable length. Subsequently, recommendations based on those made by Blacklock (1924) in the case of the village of Kaiyima were submitted, with the aim of dealing more thoroughly with the situation.

In February, 1933, Professor Gordon, of the Liverpool School Research Laboratory, in Freetown, came to Kabala. It was interesting to find that by this date there had been a big reduction in the number of *Lymnaea* and *Planorbis*, due, I believe, to some extent at least, to the first cleaning of the stream. In April, 1933, a serious effort was made to put into effect the sanitary recommendations, and at the time of leaving Kabala they had been to a great extent carried out; it will be of interest to observe and record the effect.

As has already been mentioned, three species of snail have been found in the Kabala stream. Major Connolly has identified two of them as *Lymnaea elmeteitensis* Smith and *Bulinus forskali* (Ehrn.), and the other as a *Planorbis*. It is not proposed here to go into any discussion regarding its name, as this will be done later, when, it is hoped, its anatomy will have been examined and compared with that of *P. pfeifferi* Krs. It is worthy of record that this same snail was found at Sonkonia, 20 miles north-east of Kabala.

The previous rarity of recorded cases of *S. mansoni* infection in Sierra Leone, the lack of any records of the presence of the usual snail vector at Kabala and the absence of any known focus of infection in Sierra Leone suggested the possibility that the infection at this village was of recent importation.

It is the intention of Professor Gordon, Dr. Davey and myself to publish a fuller account of this work later, giving further details and the results of animal experiments. This preliminary note serves to record the following points :—

(1) The discovery of a focus of *S. mansoni* infection at Kabala, Northern Province, Sierra Leone.

(2) The finding in the stream there of the three species of snail enumerated above, including a species of *Planorbis*.

(3) The occurrence in the *Planorbis* of what appeared to be a human type of cercaria.

(4) The initial measures adopted in an attempt to deal with the outbreak.

Due acknowledgments will be made in the later paper, but I wish here to thank Professor Blacklock for his kindly interest and advice.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE TRIBE MUSCINI, SUBFAMILY MUSCINAE, BASED ON A COMPARATIVE STUDY OF THE MALE TERMINALIA

II. THE GENUS STOMOXYS GEOFFROY (sens. lat.)

BY

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(Received for publication 24 *October,* 1933)

INTRODUCTION

The blood-sucking members of the family Muscidae belong to the subfamily Muscinae, tribe Muscini, and the genera *Musca, Stomoxys* and *Glossina.* The genus *Musca* has been dealt with in two earlier papers (1932, 1933), in which it was shown (1) that the species fall naturally into three groups on the characters of the male terminalia ; (2) that the blood-sucking habit, and with it the structure of the tearing apparatus, has evolved separately in each group ; and (3) that the viviparous habit has become established in two of the groups. The species of the world are now being revised in a series of papers in this journal.

In the present paper is recorded a similar comparative study of the male terminalia of *Stomoxys* (sens. lat.), the objects of which are (1) to discover the true affinities and relationships of the species not only to each other but to allied genera ; (2) to fix the identities of the species ; (3) to examine the status of the numerous genera erected in this group ; and lastly (4), to give a revised definition of the genus and to publish regional guides to the species of practical importance to medical and veterinary officers and to medical entomologists. The work had not progressed very far when it was realised that, owing to the extremely close similarity in the structure of the parts throughout the genus, a large number of microscopic preparations of the male terminalia, antennae, palps, proboscis and larval structures of as many species of as many genera as possible would have to be made, compared and illustrated before any conclusions could be drawn ; this has now been done.

MATERIAL ON WHICH THIS STUDY IS BASED. This study is based on the examination of the following material :—I. ADULTS. (*a*) My private collection, got together during the last 25 years and containing most of the world's species ; it is specially rich in species from the Oriental region and from China, and contains bred series and larvae. (*b*) Study of other collections, such as that of the Indian Museum, those of museums in Copenhagen, Kiel, Stockholm, Berlin, Budapest, Vienna, Paris, that of the Tropical School, Liverpool, and that in the Natural History Museum, London, which last I have now studied three times. (*c*) Study of the late Professor Bezzi's collection, especially his type material, many specimens from which he gave me ; valuable notes were made in conversation with the late Professor Bezzi, who had a very intimate knowledge of the species. (*d*) Study of the type or types of Austen, Bezzi, Bigot, Brunetti, Enderlein, Grünberg, Macquart, Malloch, Newstead, Speiser and Villeneuve. Professor Roubaud very kindly compared specimens sent to him which I considered were conspecific with *bengalensis* Picard and *indica* Picard with Picard's types, and confirmed the correctness of my identifications. Dr. J. Capra also kindly compared a specimen sent him with the type of *sitiens* Rondani, at the Museo Civico di Storia Naturale, Genoa, and his notes have been most helpful in settling the identity of Rondani's species. (*e*) Study of some recent collections kindly presented or lent for study by the following gentlemen :—(1) Dr. Green and Mr. E. P. Hodgkin, from many localities in Kuala Lumpur. This large and most valuable collection has enabled me to settle finally the identities of the species *bengalensis* Picard, *indica* Picard, *pratti* Summers and *oblongopunctata* Brunetti. (2) Mr. G. B. Purvis, F.R.C.V.S., from Alor Star, Malaya. (3) Mr. Gibbins, from Uganda. (4) Mr. A. Cuthbertson, from Southern Rhodesia ; this collection contained specimens of *uniseriatus* Malloch, a species which I have long known. (5) Mr. Carter, from Ceylon. (6) Dr. Adams, from Le Reduit, Mauritius ; this collection contained many fresh specimens of *nigra* Macquart. (7) Professor Cesar Pinto, for specimens of *lutzi* Pinto & Fonseca from Brazil. (8) Professor Roubaud, for specimens from the type material of his Ethiopian species. (9) Mr. Munro, for specimens of *uniseriatus* Malloch from South Africa. (10) Dr. P. A. Buxton, for the loan of specimens named by Summers in the collection of the London School of Hygiene. (11) Mr. W. H. Potts, for a large collection from Tanganyika, which besides containing long series of such species as *calcitrans*, *nigra*, *taeniata*, *boueti* and *brunnipes* also contains adults of *sanguinaria*, *woosnami*, *squalida* (*lutosa*), *uniseriatus* and specimens of a new species which I have long had in my collection. (12) Dr. Günther Enderlein, for the loan of the types of his *hirudo* and *equina* and microscopic preparations of the ♂ terminalia of the latter. I would like to take this opportunity of thanking these gentlemen for this material, without which this study would have been incomplete. II. MICROSCOPIC MOUNTS OF THE MALE TERMINALIA, ANTENNAE, PALPS AND PROBOSCIS. Microscopic mounts of the male terminalia, antennae, palps and proboscis of the species of the following genera have been made, compared and illustrated ; wherever possible the terminalia of at least two specimens of each species have been studied, and in many cases as many as 25.

Genus **Stomoxys** Geoffroy (sens. rest.).
bengalensis Picard
boueti Roubaud
boveri Roubaud
brunnipes Grünberg
calcitrans L.
dubitalis Malloch
indica Picard
nigra Macquart
omega Newstead
pallida Roubaud
rhodanica Roubaud
sexvittata Roubaud
taeniata Bigot

Genus **Stygeromyia** Austen.
maculosa Austen
pottsi sp.nov. (to be described later)
sanguinaria Austen

Genus **Haematobosca** Bezzi.
perturbans Bezzi
uniseriatus Malloch

Genus **Haematobia** St. Farh. & Serv.
chinensis sp.nov. (to be described later)
sanguinolentus Austen
sanguisugens Austen
squalida Grünberg
stimulans Meigen

Genus **Lyperosia** Rondani.
exigua de Meijere
irritans L.
minuta Bezzi
punctigera Austen
titillans Bezzi
thirouxi Roubaud

Genus **Neivamyia** Pinto & Fonseca
lutzi Pinto & Fonseca

Genus **Haphospatha** Enderlein
equina Enderlein

It will be seen from this list that I have studied the male terminalia of 31 (out of the possible 50) known species, and of seven genera and four subgenera as recognised by Malloch (1932) as follows :—

Tribe Stomoxyidini Malloch.		
Genus *Stomoxys* Geoffroy (sens. rest.)	13	species
„ *Bruceomyia* Malloch	1	„
„ *Neivamyia* Pinto & Fonseca	1	„
Tribe Haematobiini Malloch.		
Genus *Stygeromyia* Austen	3	„
„ *Bdellolarynx* Austen, subgenus *Bdellolarynx* Austen	3	„
„ „ „ *Lyperosiops* Townsend	3	„
„ *Haematobosca* Bezzi	1	..
„ *Haematobia* F. & S., subgenus *Haematobia* F. & S...	2	„
„ „ „ *Glossinella* Grünberg	4	„

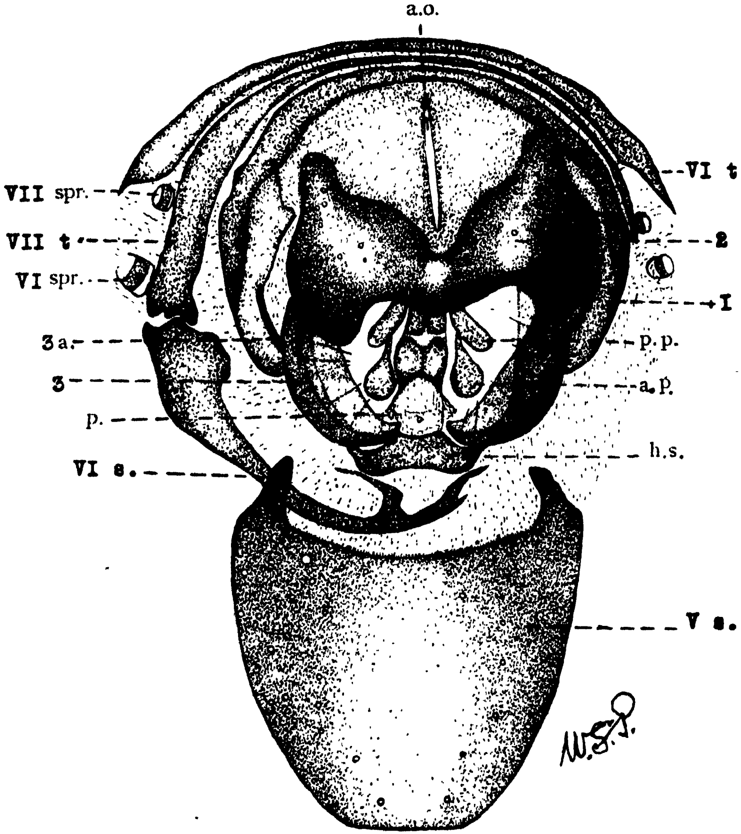

FIG. 1. Male terminalia of *calcitrans*. Vs., VIs.—Fifth, sixth sterna ; VIspr., VIIspr.—Sixth, seventh spiracles ; VIt., VIIt.—Sixth, seventh terga ; 1.—Tenth tergum ; 2.—Anal cerci ; 3.—Proximal segment of ninth coxite ; 3a.—Distal segment of same ; *a.o.*—Anal opening ; *a.p.*—Anterior paramere ; *h.s.*—Horned sclerite ; *p.*—Phallosome ; *p.p.*—Posterior paramere.

SHORT DESCRIPTION OF THE MALE TERMINALIA OF STOMOXYS CALCITRANS

The male terminalia of *Stomoxys* (sens. lat.), as exemplified by those of *calcitrans*, are built on the same plan as those of *Musca*. There are ten abdominal segments, seven of which bear spiracles as in *Musca*, so that it is not necessary here either to describe or to depict them ; the eighth segment is wanting. I would again emphasize the importance of locating the spiracles and the anal opening as the only certain means of understanding the segmentation of the abdomen and the homologies of the parts. The terminalia as seen from the ventral side are illustrated in fig. 1 ; they are drawn from a specimen dissected off and mounted without compression. As the ninth tergo-sternum in the specimen is lying behind the anal cerci it is not illustrated, in order to avoid complicating the drawing. As in *Musca*, the sixth tergum is a short, rather narrow arched plate, concealed under the posterior edge of the fifth. The seventh is similar, but wider, and is also slightly asymmetrical, the left side being more strongly developed and articulating with the sixth sternum. The sixth sternum is also asymmetrical, and is a bent rod-like sclerite, extending forwards and inwards to end just internal to the right process of the fifth sternum. Close to its articulation with the seventh tergum it has a plate-like expansion directed downwards and slightly inwards. At its inner end it is attached to the arthrodial membrane at the distal end of the fifth sternum ; this has two strongly chitinised areas which vary in shape and extent in the different species but are present in all so far examined.

The ninth segment consists of the so-called tergo-sternum which bears the phallosome and parameres. The tenth or anal segment is represented by its tergum, an arched plate directed posteriorly, forming the rounded apex of the abdomen. It is incised postero-ventrally by a large oval incision extending well back so that the postero-dorsal part is much reduced in width. The incision is closed by a stout membrane which is attached along its margins. Extending antero-posteriorly almost the whole length of the membrane there is a narrow slit, the anal opening (fig. 1, *a.o.*) directed ventrally ; anteriorly the incision is closed by the anal cerci (2 in fig. 1). On each side anteriorly the tergum consists of a deep plate, the upper and lower margins of which form a short process directed inwards, and

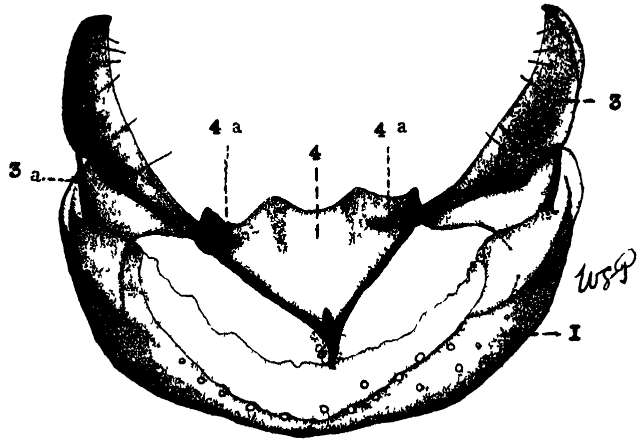

FIG. 2. Dissection, showing tenth tergum, ninth tergo-sternum and ninth coxites of *calcitrans*, drawn apart, the anal cerci have been removed 1 —Tenth tergum, 3.—Proximal segment of ninth coxite ; 3a —Distal segment of same , 4 —Ninth tergo-sternum ; 4a.—posterior process of same

articulating in a notch in the side of the ninth tergo-sternum (fig. 2). The tenth sternum either is wanting or is represented by the membrane dividing the space enclosed by the tergum, and so separating the alimentary tract from the genital atrium. This membrane is attached to the back of the phallosome and the processes of the ninth tergo-sternum.

ANAL CERCI. 2 in fig. 1 ; Fig. 3, *a*.—The anal cerci are attached to the anterior end of the tenth tergum, as in *Musca*, and close the incision. In *Stomoxys* they are fused in the middle line, and cannot, therefore, be separated without being torn apart ; in *Musca*, on the other hand, they are lightly attached to each other by a slip of chitin, and can readily be separated. Each cercus is a short wing-like plate, the apex of which is directed posteriorly and is convex externally and concave internally. The free margin is reinforced by an infolding or thickening of the chitin which extends round to the point of articulation with the proximal segment of the ninth coxite. The outer end of the free margin of each is rounded in *calcitrans* and its close allies, the inner end is fused with that of its fellow, so that the two form a rounded median projection (2 in fig. 1) ; between the two ends the free margin is slightly incised. Postero-laterally about half the length of the cercus, the chitinous infolding on the inner side forms a projection which articulates against the lower margin of the proximal segment of the ninth coxite. Posteriorly each cercus

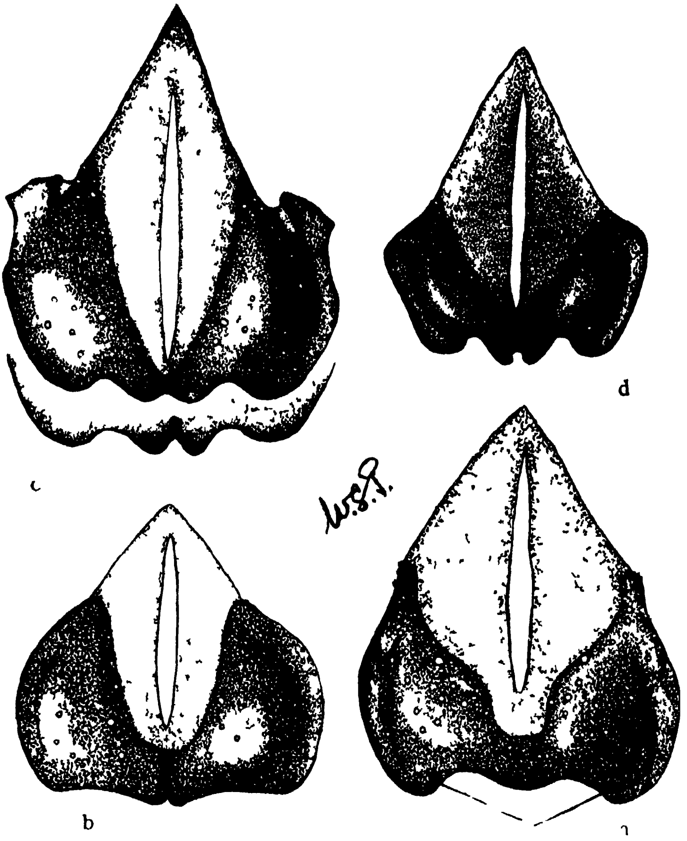

FIG. 3 *a.*—Anal cerci of *calcitrans*, note how the inner ends are completely fused; *b.*—same of *stimulans*, note how inner ends are separated by incision, and then appear as nipples, *c*—same of *maculosa*, note incision and nipple-like inner ends, *d*—same of *irritans*, note the deep notch and nipple-like inner ends.

narrows to a blunt point (the apex or tip of the wing), which is attached to the membrane. The inner margin, as well as the outer surface, is well supplied with strong bristles and hairs. NINTH COXITE. 3, 3*a* in figs 1, 2; fig. 4, *d*. In the space between the outer edge of each cercus and the anterior extension of the tenth tergum, there is located in the membrane the narrow, hairy distal segment of the ninth coxite (3*a*); it is attached to the side of the cercus by membrane. Anteriorly it articulates with the side of the proximal segment, and posteriorly it is fused with the lateral chitinous extension from the lower margin of the anterior end of the tenth tergum. The proximal segment (3 in figs. 1, 2) is shaped like the human hand with the fingers closed together, and, like that of *Musca*, is convex externally and concave internally; each lies antero-posteriorly just internal to the anterior part of the tenth tergum. The distal or free end of each is directed inwards towards the middle line, and ends in a hook-like blunt point. The proximal end narrows to a strong rod directed slightly inwards, and is attached to the posterior surface of the end of the postero-lateral process of the ninth tergo-sternum; this attachment allows of a wide range of movement (fig. 5, *c*). The ventro-lateral margin is expanded and consists of lighter chitin. The inner surface is supplied with a few strong stiff hairs, the outer with small sensory spines. As in *Musca*, each consists of two plates of chitin fused together; they form admirable claspers for grasping the end of the ovipositor.

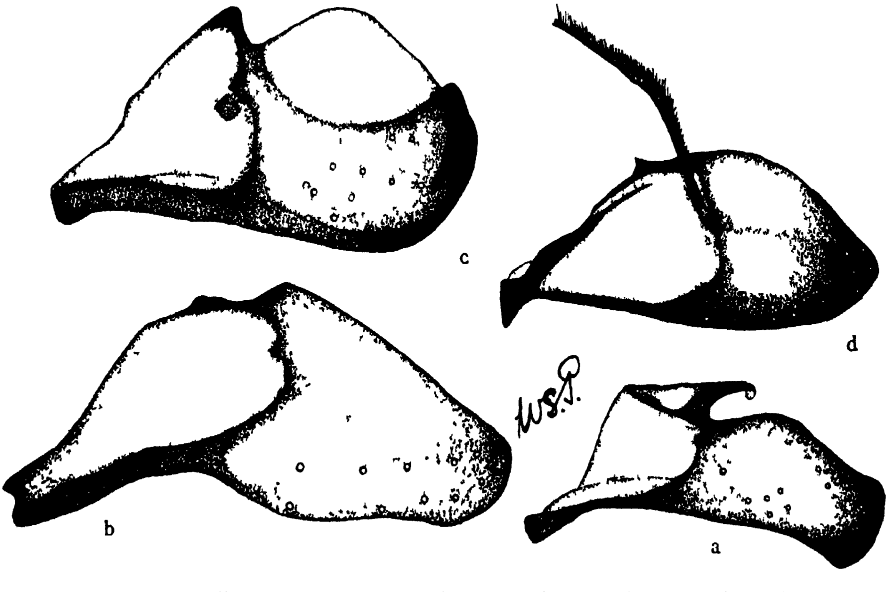

FIG. 4. *a.*—Proximal segment of ninth coxite of *irritans*; *b.*—same of *maculosa*; *c*—same of *stimulans*; *d*—Proximal and distal segments of same of *calcitrans*.

NINTH TERGO-STERNUM. Fig. 5, *c*. The ninth tergo-sternum is a strongly arched, somewhat V-shaped plate, its convex surface directed downwards and slightly forwards in the resting position. Its outer margins are formed of strong chitinous rods which, meeting dosally, end in a stout blunt point; they curve behind towards the middle line, ending freely near each other; the proximal segment of each ninth coxite is attached to the posterior process of the ninth tergo-sternum of its side, as shown in figs. 2, 5, *c*. At the lower (broad) end, the chitin is raised on each side of the middle line where each paramere is attached, the phallosome resting between them. The membrane forming the pocket for the phallosome (when folded in the position of rest) is, as in all these muscids, attached along the apex and sides of the ninth tergo-sternum on the one hand, and along the ends of the fifth and sixth sterna on the other. The shape of the tergo-sternum varies in the different species.

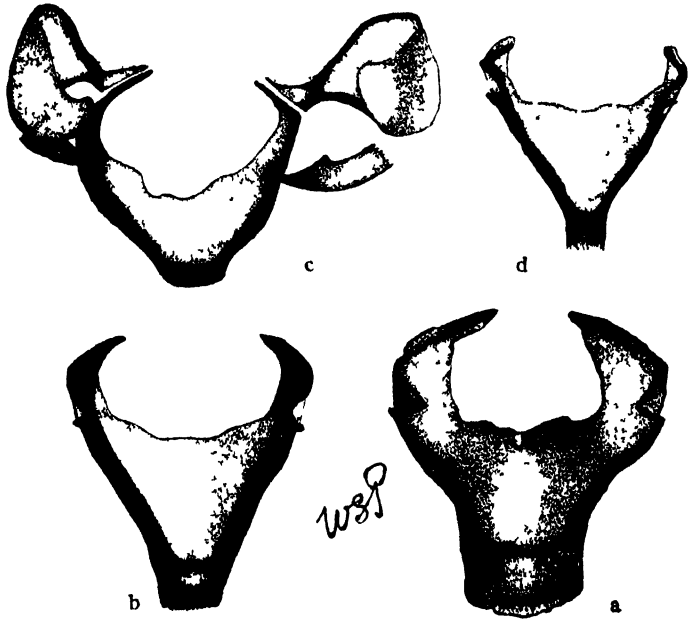

FIG. 5. *a*.—Ninth tergo-sternum of *maculosa*, note the wide postero-lateral processes; *b*.—same of *stimulans*; *c*.—same of *calcitrans*, also showing attachment of proximal segment of ninth coxite, *d*.—same of *irritans*.

PHALLOSOME. Fig. 6, *a*. The phallosome is partly chitinous and partly membraneous. The proximal chitinous part is a short tube consisting of two plates folded round and united posteriorly in the middle line; postero-laterally each has a strong projecting chitinous process on which each half of the paramere rests (' branche latérale du pénis ' of Dinulescu). The proximal chitinous tube is attached posteriorly to the distal chitinous portion by chitin, and anteriorly by membrane, as is well seen in fig. 6, *a*. The distal chitinous part is shaped like a funnel, the anterior wall being longer than the posterior, the two folded round forming the tube and attached to each other posteriorly. The membraneous part is relatively short and is attached to the wide end of the funnel; the ejaculatory duct opens on it. Attached to the free end of the anterior wall of the distal chitinous tube, and at right angles to it, there is a broad, deeply pigmented sclerite, each side of which is produced into a strong spine-like process; there is sometimes a second smaller spine above the larger one in *calcitrans*, and in other species it is always present. This sclerite of the phallosome is extremely characteristic of all the species of the genus *Stomoxys* (sens. lat.), and so far I have not seen it on the phallosome of the species of any other genus. Its appearance is well seen in fig. 6, *d*. It will be referred to as the horned sclerite. The phallosome of the 31 species so far examined only differs in minute details and in all the posterior process is *wanting*. The apodeme of the phallosome when viewed from the front is seen to consist of a Y-shaped sclerite, the two arms of the Y consisting of strongly chitinised rods united by lighter chitin, which extends upwards between the two anterior parts of the paramere, and is united by membrane to the bar joining them; at the distal ends the rod-like arms unite to form a short bluntly-pointed rod. All these details are well seen in the various drawings in fig. 6.

PARAMERE. Fig. 6, *a*, *b*, *d*, *f*, *g*. Each paramere consists of two *distinct* parts joined only by membrane to each other and to the side of the phallosome. The antero-basal part of the posterior, and the postero-basal part of the anterior, portion each rests against the projecting process on the lower tubular part; this is well seen in fig. 6, *e*. The anterior part of the paramere is an upstanding process formed, as in *Musca*, of a folded sclerite, and is, therefore, hollow. Its appearance varies according to the position from which it is viewed (fig. 6, *b*, *f*, *h*). The upper surface is somewhat flat and is joined to its fellow anteriorly by a short chitinous bar (fig. 6, *h*). In *calcitrans* it is armed with one rather long and 1-3 short bristles on its postero-lateral face. It varies slightly in size and shape in the different species. The posterior portion of the paramere is also a hollow raised process, broad when seen from the side, and always narrowing distally, the end bluntly pointed, bent forwards, and in *calcitrans* and its near allies always *without* any tuft of hairs; in *calcitrans* and its near allies it commonly has a shoulder on it near the

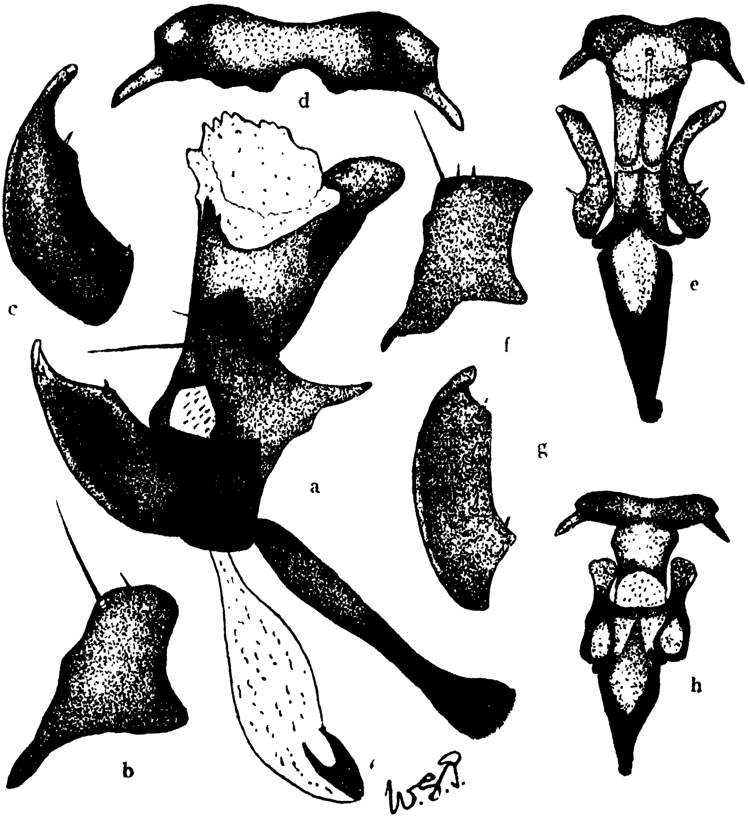

FIG. 6. *a.*—Phallosome and paramere of *calcitrans* (Liverpool) ; *b.*—Right anterior paramere of same (Ceylon) ; *c.*—Right posterior of same (Liverpool) ; *d.*—Front view of horned sclerite of same ; *e.*—Posterior view of phallosome and parameres of same (Liverpool) ; *f.*—Right anterior paramere of same (Liverpool) ; *g.*—Right posterior paramere of same (Ceylon) ; *h.*—Phallosome and parameres from front of same (Liverpool).

distal end. It is always armed with 1-4 (sometimes 6) short sensory (?) spines. The illustrations of the two parts of the paramere in fig. 6 explain their shape, etc., better than words can do.

STERNA. Fig. 7, *d.* The first sternum is broadly half-moon-shaped and has blunt ends. The second is closely attached to it, and is shield-shaped ; it has two narrow anterior processes joined by a delicate bar, which usually lies behind the posterior end of the first sternum. The third is narrowly elongated and has a short, dark chitinous anterior prolongation. The fourth is very similar, but usually longer and narrower. The fifth (fig. 7, *d*) is elongated and, excluding the posterior processes, is about as long as broad. It has

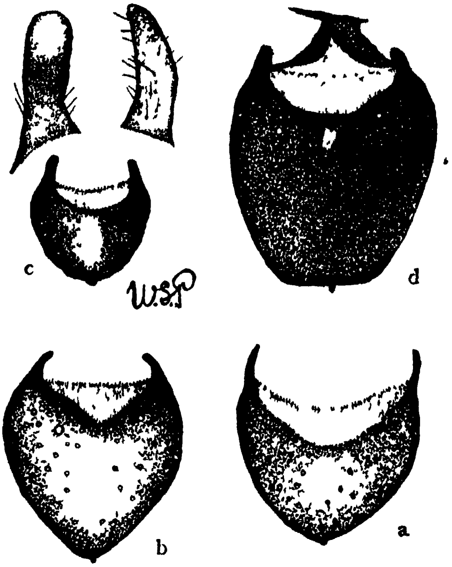

FIG. 7. *a.*—Fifth sternum of *maculosa* (Bengal), *b*—Fifth sternum of *stimulans* (England); *c.*—Fifth sternum of *irritans* (Italy) and posterior processes, enlarged; *d*—Fifth sternum of *calcitrans* (Liverpool), drawn to same scale

a small, dark rod-like chitinous process at the middle of the posterior end. The posterior processes are fairly long and broad, and are as a rule minutely serrated (only seen under a high magnification).

PREVIOUS DESCRIPTIONS OF THE MALE TERMINALIA OF STOMOXYS (SENS. LAT.)

There are two recent descriptions of the male terminalia of *Stomoxys* (sens. lat.), one by Dinulescu (1930) of those of *calcitrans*, the other by Cesar Pinto (1932) of those of *calcitrans*, *nigra* and *lutzi*. The former author evidently overlooked Awati's paper (1916), and, failing to recognise the importance of locating the spiracles and the anal opening as the only certain guides to the segmentation of the abdomen and the homologies of the parts, has, as a result, given erroneous names to them; it is necessary to correct these. Dinulescu's eighth tergum is the tenth, his 'lamelle génitale' is the fused anal cerci, his 'forceps externes' is the proximal segment of the ninth coxite, his 'sternite génitale' is the ninth tergo-sternum, his 'première et deuxième branche du sternite génitale' together form the postero-lateral process of each side of the ninth tergo-sternum; there seems

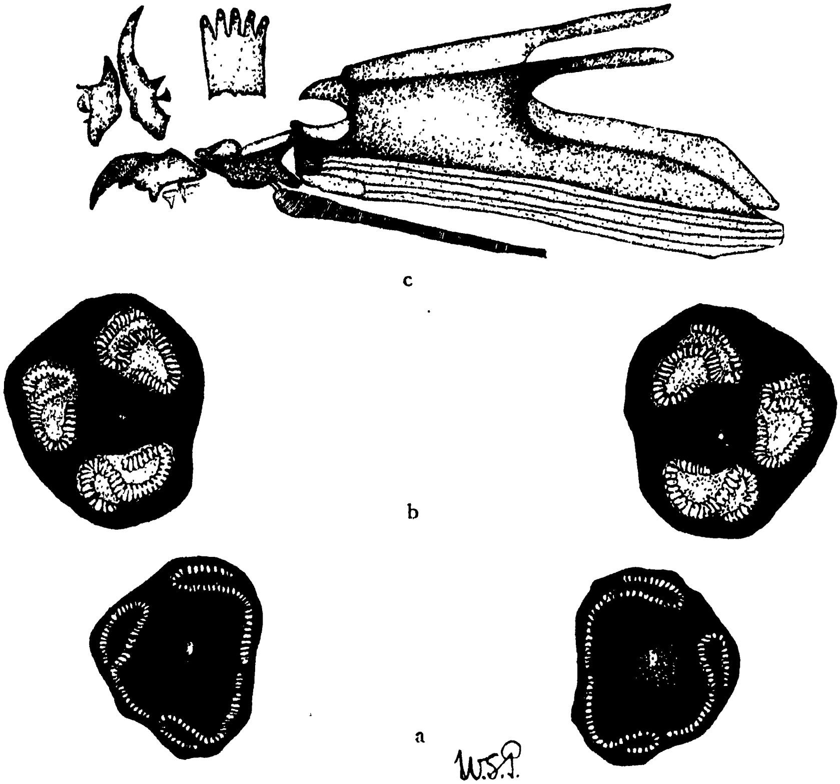

FIG. 8. *a.*—Posterior spiracles of *stimulans* (England) ; *b.*—Same of *calcitrans* (England) ; *c.*—Cephalopharyngeal skeleton of third larva of *calcitrans*, also enlarged oral hooks and anterior spiracle ; spiracles drawn to same scale.

to be some confusion here, for the ninth tergo-sternum has only one process on each side, as shown in the illustrations (figs. 2, 5). Dinulescu's 'forceps internes inférieurs' is the anterior part of the paramere, and his 'forceps internes supérieurs,' the posterior portion of the same ; and lastly his 'plaque prégénitale' is the fifth sternum.

LARVAL CHARACTERS IN STOMOXYS (sens. lat.). I have studied the breeding habits and the larvae of many species of this genus, and from observations made in the field, as well as from dissections, I believe it can safely be concluded that all the species are oviparous in habit. As the eggs are always laid singly, a few at a time, and are scattered in the larval foodstuff, the larvae are, as a rule, difficult to find, even in localities where the adults may be numerous. THIRD LARVA. CEPHALOPHARYGEAL SKELETON. The third larva is structurally similar to that of *Musca*, but the anterior end is much more

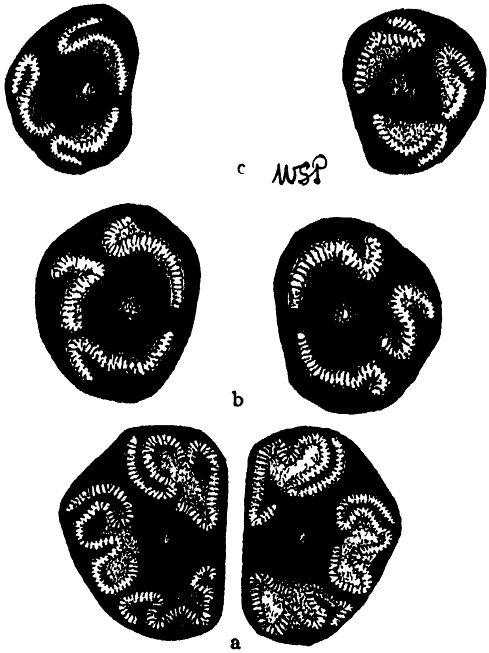

FIG. 9. *a*.—Posterior spiracles of third larva of *exigua* (China); *b*.—Same of *chinensis*; *c*.—Same of *sanguisugens* (Kasauli, India); drawn to same scale.

attenuated. The cephalopharyngeal skeleton of the third larva of *Stomoxys calcitrans* is illustrated in fig. 8, *c*. The most important differences between it and that of the third larva of *Musca* are as follows:—1. The small size of the left oral hook, which is much smaller than that of the third larva of *Musca*. 2. The longer, and more boat-shaped, hypostomal sclerite. 3. The longer pharyngeal sclerite, especially the cornua—the ventral one without the hump which is present in the third larva of *Musca*. POSTERIOR SPIRACLES. The posterior spiracles of species of the ***stimulans***, ***irritans*** and ***calcitrans*** groups are illustrated in figs. 8, 9; I have not yet had an opportunity of studying the posterior spiracles of the larva of any species of the ***maculosa*** group. On comparing these illustrations it will be noted that except for minor differences they are built on the same plan. In the ***calcitrans*** group the plates are usually relatively small and widely separated; in most of the species of the ***stimulans*** group they are also widely separated (but not so much as in ***calcitrans***), whereas in the ***irritans*** group they are closer together. The plates in all are more or less D-shaped, tending to become rounded ; the three breathing slits are sinuous, long (longest in the *irritans* and shortest in the *calcitrans* group), narrow, and arranged as shown in the illustrations ; the button is central in position. There are seldom more than 5-7 finger-like processes on the anterior spiracle. The puparium is always dark brown.

THE NATURAL GROUPING AND RELATIONSHIPS OF THE SPECIES

The comparative study of the male terminalia of 31 (out of a possible 50) species of *Stomoxys* (sens. lat.) has revealed the fact that they fall naturally into four groups, which I propose naming the *stimulans*, *irritans*, *maculosa* and *calcitrans* groups, respectively, using the name of the oldest species in each, as I have already done in the groups of *Musca*. In order to understand this grouping and the relationships of the species to each other, it will only be necessary here to note in what respects the parts of the male terminalia of the type of each group differs from those of the other three ; there is no necessity to describe the male terminalia of *stimulans*, *maculosa* and *irritans* in detail. The important differences, on which the groups and relationships are based, are to be found to some extent in the structure of the phallosome, mainly, however, on that of the paramere, and especially its posterior portion, the structure of the anal cerci, that of the proximal segment of the ninth coxite, that of the ninth tergo-sternum, and to a less extent on that of the fifth sternum.

I. Stimulans Group. Fig. 10. The phallosome, paramere and parts of these of *stimulans* Meigen, the type of the group, are illustrated in fig. 10. The anal cerci are illustrated in fig. 3, *b*, the proximal segment of the ninth coxite in fig. 4, *c*, and the ninth tergo-sternum in fig. 5, *b*. The following differences in the above structures are characteristic of all the species of this group so far examined :—
1. Phallosome, Paramere. Fig. 10, *a*. There is very little difference between the structure of the phallosome and that of the anterior part of the paramere of this species and that of those of the types of the other three groups ; what differences there are will be noted under each. The posterior part of the paramere is, however, in the majority of the species narrower than that of *calcitrans* and its allies, the distal narrow portion is usually long and bent, and the end is *always* armed

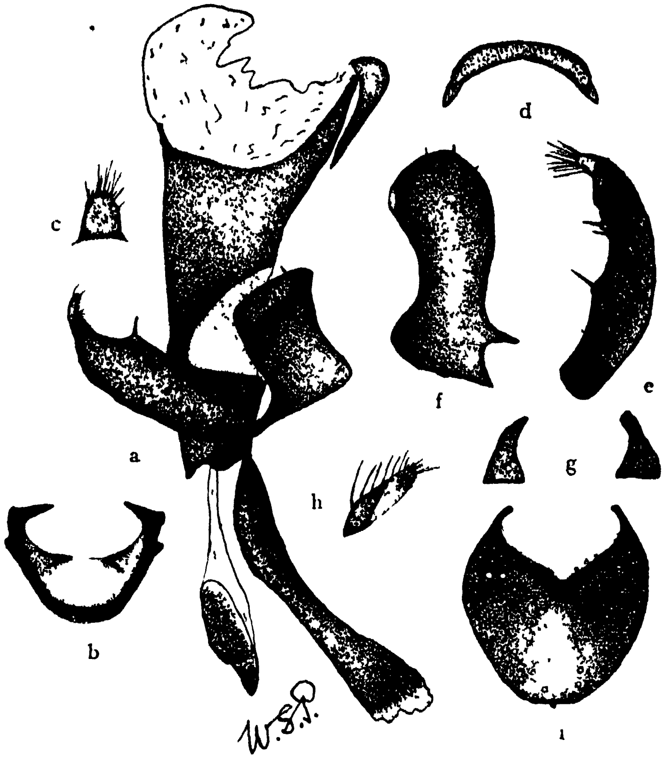

FIG. 10. *a.*—Phallosome and one paramere of *stimulans*, *b.*—Ninth tergo-sternum of same ; *c.*—End of posterior paramere of same, showing group of microscopic hairs ; *d.*—Front view of horned sclerite of same ; *e.*—Left posterior paramere of same, showing spines and microscopic hairs at end ; *f.*—Left anterior paramere of same ; *g.*—Ends of postero-lateral processes of fifth sternum of same ; *h.*—Another view of end of posterior paramere, showing microscopic hairs ; *i.*—Fifth sternum of same.

with a group (fringe) of delicate microscopic hairs, the number and arrangement of which varies in the different species ; this is the most important group character. 2. ANAL CERCI. Fig. 3, *b.* Although the anal cerci are fused along the middle line, the junction being indicated by a dark median line of varying length, the free margin of the inner end of each cercus is not fused with that of its fellow, there being an incision of varying depth (depending on the species) separating the inner ends, which may then project slightly as nipples. 3. PROXIMAL SEGMENT OF THE NINTH COXITE. Fig. 4, *c.* The proximal segment of the ninth coxite is large, the distal end is broadly rounded, and the anterior edge has a raised, rounded, more distal part, as shown in the illustration. 4. NINTH TERGO-STERNUM.

Fig. 5, *b*. The ninth tergo-sternum is V-shaped and, as in *Musca*, consists of a single sclerite ; it lies in the middle line, almost in the vertical position. The arms of the V consist of strong chitinous rods, which meet dorsally and end in a stout blunt point ; each postero-lateral process is a stout, broad, bluntly-pointed bar bending round well behind the tergum towards its fellow of the opposite side.

5. Fifth Sternum. Fig. 7, *b*. The fifth sternum is usually heart-shaped and varies in size according to the species ; the posterior end is usually bluntly pointed, and the antero-lateral processes are rather long, not markedly narrow, bluntly pointed, and as a rule unserrated. The following twelve species, the male terminalia of which have so far been examined, belong to this group :—

chinensis sp.nov.
equina Enderlein
lutzi Pinto & Fonseca
perturbans Bezzi
punctigera Austen
sanguinolentus Austen
sanguisugens Austen
squalida Grünberg
stimulans Meigen
thirouxi Roubaud
titillans Bezzi
uniseriatus Malloch

In the case of ***stimulans***, ***sanguisugens***, ***chinensis***, ***squalida***, ***perturbans***, ***uniseriatus***, ***equina***, ***titillans*** and ***thirouxi***, the palps are dilated and are about as long as the proboscis ; in the resting position they lie closely applied to its upper surface (fig. 15). In ***lutzi*** and ***punctigera***, the palps are also about as long as the proboscis, but are of uniform diameter throughout (fig. 13, *b*, *e*). In the case of ***stimulans***, ***sanguisugens***, ***sanguinolentus***, ***chinensis***, ***squalida*** and ***lutzi***, both surfaces of the arista are armed with spinulae, the lower usually having at least three hairs, and in some of the species more than three. In ***perturbans*** and ***uniseriatus***, both surfaces of the arista are also haired, the lower usually having only two hairs, but there may be three. In ***punctigera***, both surfaces are usually haired ; the lower may only have one hair, sometimes two, and may even be bare. In ***titillans***, ***equina*** and ***thirouxi***, the arista has spinulae only on the upper surface, the lower always being bare. After comparing the terminalia of ***titillans*** with that of ***equina***, I am inclined to the view that they are identical. I believe that the following nine species will be found to have microscopic hairs at the end of the posterior part of the paramere, and will, therefore, belong to this group :—

atrifrons Malloch
atripalpis Bezzi
hirudo Enderlein
hirtifrons Malloch
latirostris Enderlein
potrix Enderlein
praedatrix Enderlein
shillingsi Grünberg
spinigera Malloch

I hope shortly to describe *chinensis*, a new species discovered in 1926 at Tsinan, Shantung, North China.

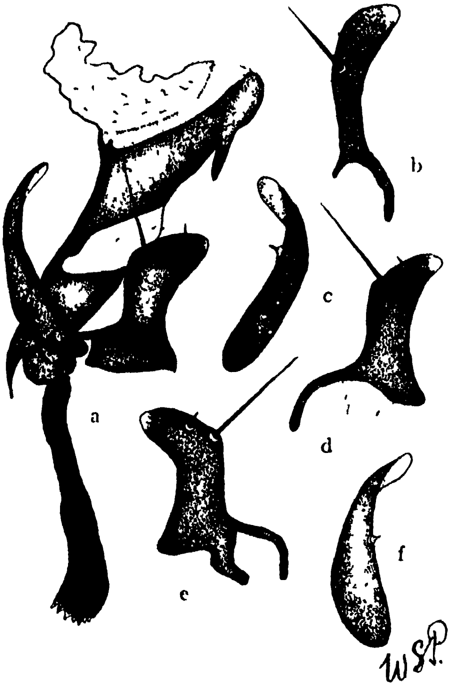

Fig. 11 *a*.—Phallosome and one paramere of *irritans* (England); *b*.—Posterior view of anterior paramere of same (Italy, Bezzi material), *c*.—Left posterior paramere of same (Italy, Bezzi material); *d*.—Right anterior paramere of same (England); *e*.—Left anterior paramere of same (Italy, Bezzi material); *f*.—Right posterior paramere of same (England).

II. Irritans Group. Fig. 11. The phallosome and paramere and parts of these of *irritans*, the type of this group, are illustrated in fig. 11. The anal cerci are illustrated in fig. 3, *d*; the proximal segment of the ninth coxite in fig. 4, *a*; and the ninth tergo-sternum in fig. 5, *d*. 1. Phallosome, Paramere. Fig. 11, *a*. The phallosome is, for the size of this species, rather long, especially the proximal chitinous tubular part; the distal chitinous portion is long and narrow, and is like that of *maculosa* (fig. 12, *a*). The horned sclerite often has an additional spine or process on each side. The anterior part of the paramere is long, narrowed in the middle, and the distal end is somewhat flattened; it has in this species a rather long narrow posterior extension, which is also present in the two other species.

The posterior part of the paramere is long and in side view appears to be narrower in its distal half, but when seen from in front or behind it appears to be of almost uniform diameter throughout. The distal end is slightly expanded and is usually more membraneous ; the distal end is never armed with microscopic hairs, but has the usual sensory spines. 2. ANAL CERCI. Fig. 3, *d*. The anal cerci are fused, the junction being of less dense, rather membraneous chitin, so that the two can easily be torn apart. In *irritans* and *exigua*, the inner ends are free, projecting as nipples, and are separated by a deep notch ; in *minuta* there is no such notch. There is a marked incision between the two ends of each cercus, so that the outer ends also project as broad nipples, as do those of the species of the *maculosa* group (fig. 3, *c*.). 3. PROXIMAL SEGMENT OF THE NINTH COXITE. Fig. 4, *a*. The proximal segment of the ninth coxite is long and narrow, and is very characteristic of the species of this group. The distal end is broadly rounded, and there is a long process bent hook-like on the anterior border near the base, evidently homologous with the similar but smaller process on the same segment of *stimulans* and *maculosa* ; it is separated from the distal rounded part by a deep incision ; the process is somewhat similar in *exigua*, but is much shorter in *minuta*. 4. NINTH TERGO-STERNUM. Fig. 5, *d*. The ninth tergo-sternum is relatively small, but is very similar to that of *stimulans*. 5. FIFTH STERNUM. Fig. 7, *c*. The fifth sternum is rather small, almost as long as wide, the postero-lateral processes long, broad, bluntly-pointed and unserrated. At present I know of only three species which belong to this group, as follows :—

exigua de Meijere
irritans L.
minuta Bezzi

Exigua is the well-known bovine pest and, in view of its very wide distribution in the Oriental and Australasian regions, in China as far north as Peking, and in Iraq, and as it is a variable species, I have compared the male terminalia of specimens from many localities in these regions, and find that they are all identical. This species, then, in addition to being found throughout the Oriental and Australasian regions, is widely distributed in the Palaearctic region.

III. MACULOSA GROUP. Fig. 12. The phallosome, paramere and parts of these of *maculosa* Austen, the type of the group, are illustrated in fig. 12. The anal cerci are illustrated in fig. 3, *c*, the

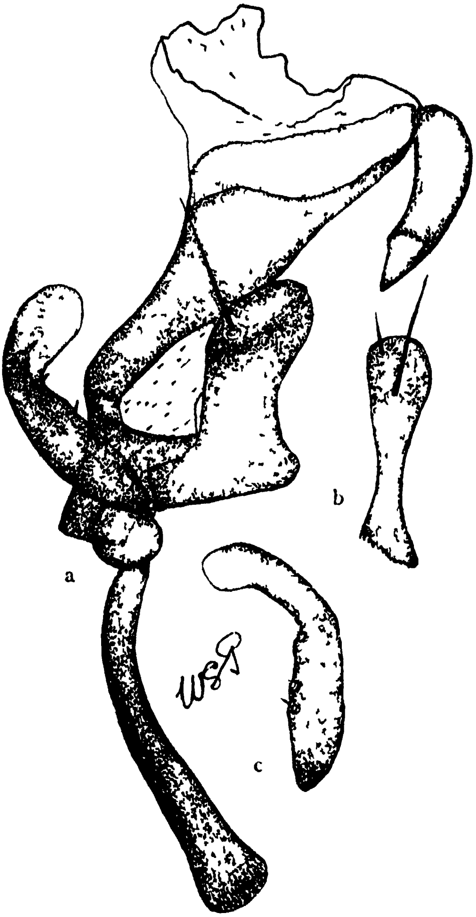

FIG 12 *a.*—Phallosome and paramere of *maculosa* (Bengal) ; *b.*—Posterior view of anterior paramere of same (Madras) , *c* —Left posterior paramere of same (Madras).

proximal segment of the ninth coxite in fig. 4, *b*, and the ninth tergosternum in fig. 5, *a*. 1. PHALLOSOME, PARAMERE. Fig. 12, *a*. The phallosome (especially its horned sclerite) is larger than that of either *calcitrans* or *stimulans*. The distal tubular chitinous portion is rather long and narrow, and though much longer and broader is somewhat like that of *irritans* (fig. 11, *a*) ; it is on the whole narrower and longer than that of most of the species of the *calcitrans* and *stimulans* groups so far examined. The anterior part of the paramere (fig. 12, *a*, *b*) is a long stout process ; the posterior portion (fig. 12, *a*, *c*) is of about uniform diameter throughout, and narrower than the corresponding part of the paramere of the species of the *calcitrans* group so far examined, and also of most of those of the *stimulans* group; its distal free end is *always* without hairs and, in the three species of the four known to me, has an outer membraneous expansion which appears to be characteristic of the group (see fig. 12, *a*, *c*). 2. ANAL CERCI. Fig. 3, *c*. The anal cerci are fused in the middle line, the free margin of the inner end of each is, however, separated from that of its fellow by a well-marked incision, so that the inner ends project freely as nipples; there is, in addition, rather a deep incision separating the inner end of each cercus from the outer, so that the latter then also projects to form a broad nipple-like process. The fused anal cerci of the species of this group thus bear a superficial resemblance to those of some species of the *lusoria* group of *Musca* - for instance, those of *lusoria* and *spinohumera* (see *Musca*, pt. 1, fig. 15, *b*, p. 381, 1932). This appearance is extremely characteristic of the anal cerci of the three species of the *maculosa* group so far examined. 3. PROXIMAL SEGMENT OF THE NINTH COXITE. Fig. 4, *b*. The proximal segment of the ninth coxite is a stout rather long sclerite, longer than that of any other of the type species of the three other groups. Although narrower than that of *stimulans*, it shows some structural similarity; it is also in some respects not unlike that of *irritans*. Its free extremity is rather bluntly rounded, the anterior edge has two rather shallow raised areas separated by a depression, the remaining part sloping gently to the end. 4. NINTH TERGO-STERNUM. Fig. 5, *a*. The ninth tergo-sternum is a heavily chitinized, broad sclerite with a broadly-rounded dorsal end and strongly developed, broad, pointed, converging postero-lateral processes (fig. 5, *a*); its structure clearly suggests a relationship with the ninth tergo-sternum of *stimulans*. All the three species of this group, the male terminalia of which have so far been examined, have each an almost exactly similar tergo-sternum. 5. FIFTH STERNUM. Fig. 7, *a*. The fifth sternum is heart-shaped, the postero-lateral processes rather long, narrow, and usually unserrated, and are like those of the fifth sternum of *stimulans*. At the present time I know of four species of this group, all the adults of which I have studied; I have not so far had an opportunity of examining the male terminalia of *woosnami*; the species are the following: - -

maculosa Austen	*sanguinaria* Austen
pottsi sp.nov.	*woosnami* Austen

I have in an earlier paper (1914) recorded the occurrence of *maculosa* in India, where I now know it is widely distributed ; it is mainly crepuscular in habit and, when feeding and at rest, crosses its wings scissor-fashion. With the aid of the recent additional material of Ethiopian species sent me by Mr. Potts from Tanganyika, I have been able to examine the male terminalia of *sanguinaria.* I have long had a new Ethiopian species in my collection, but have hesitated to describe it until I have had an opportunity of examining more material and especially the male terminalia of *sanguinaria.* In the collection from Tanganyika, Mr. Potts has sent me some additional specimens of this new species, and I have great pleasure in naming it after him ; I hope to describe it soon. The collection also contained two females of *woosnami,* only the male of which has so far been known.

It is interesting to note that in all four species of this group the palps are dilated and lie closely applied to each other on the *upper* surface of the short proboscis. In all, the arista has spinulae only on the upper surface. In all, cell R_5 is narrowly open, veins R_{4+5} and M_{1+2} converging towards each other at the wing margin.

IV. CALCITRANS GROUP. Fig. 6. The phallosome, paramere and parts of these are illustrated in fig. 6. The anal cerci are illustrated in fig. 3, *a*, the proximal segment of the ninth coxite in fig. 4, *d*, and the ninth tergo-sternum in fig. 5, *c*. 1. PHALLOSOME, PARAMERE. Fig. 6, *a*. There are no particular differences to note in the structure of the phallosome of *calcitrans* as compared with that of *stimulans*, *maculosa* and *irritans* ; the important differences have been noted in the case of the others. The anterior part of the paramere is rather broad and long when seen from the side, and has usually two, sometimes three, bristles ; it varies a little in length and shape in the different species. The posterior part is broad in side view, narrowing towards the end, which is bent forwards, giving the appearance of a shoulder (fig. 6, *a*, *c*, *g*) ; the length of the distal end varies according to the position from which it is viewed (fig. 6, *c*, *g*). In most of the species of the group the posterior part is very similar to that of *calcitrans*, but may be narrower ; it is never armed with hairs at the distal end. 2. ANAL CERCI. Fig. 3, *a*. The anal cerci are completely fused, the inner ends together forming a single rounded projection ; this appearance is very characteristic of the species of this group. This median rounded part is separated from the outer end of each cercus by an incision of varying depth in the different species, so that the outer end may project to a varying extent. 3. PROXIMAL SEGMENT OF THE NINTH COXITE. Fig. 4, *d*. The proximal segment of the ninth coxite is relatively shorter than that of the type of the three other groups, and is more pointed at the free end, which is somewhat bent in. The anterior border has a small process, and then slopes round to the distal end. 4. NINTH TERGO-STERNUM. Fig. 5, *c*. The ninth tergo-sternum is somewhat U-shaped, the postero-lateral processes rather long, slender and curved well round posteriorly. 5. FIFTH STERNUM. Fig. 7, *d*. The fifth sternum is longer than broad, the posterior end broadly rounded, and the postero-lateral processes relatively short, broad and usually finely serrated. The following species, the terminalia of which have been examined, belong to this group :-

bengalensis Picard
boueti Roubaud
bouveri Roubaud
brunnipes Grünberg
calcitrans L.
dubitalis Malloch
indica Picard
nigra Macquart
omega Newstead
pallida Roubaud
rhodanica Roubaud
sexvittata Roubaud
taeniata Bigot

I have yet to examine the male terminalia of the following species : —

bilineata Grünberg
inornata Grünberg
ochrosoma Speiser
pulla Austen
sitiens Rondani
transvittata Villeneuve
triangularis Brunetti
varipes Bezzi

In all the species of this group, the palp is short, much shorter than the proboscis (which is exceptionally long and narrow), and is undilated ; it should be noted, however, that the length of the palp varies in the different species. The arista has spinulae only on the upper surface ; cell R_5 is rather widely open.

It will be remembered that, as a result of the comparative study of the male terminalia in *Musca* (1932), no difficulty was experienced in reaching a conclusion as to the affinities of the species, the structure of the posterior part of the paramere alone clearly defining the groups and indicating the relative position of each within the genus. In *Stomoxys* (sens. lat.), on the other hand, it has been necessary to study and compare in great detail all the parts of the male terminalia, in order to discover the natural relationships and grouping of the species. The very close similarity of the parts in the species has, however, rendered it impossible at present to reach a final conclusion as to the relative position of the groups within the genus. The structure of the anal cerci, that of the proximal segment of the ninth coxite, that of the posterior spiracles of the larva, and perhaps the persistance of pseudotracheae on the labella, suggest that the species of the *stimulans* group are the nearest relatives of *Musca.* On the other hand, the three species of the *irritans* group known at present also possess certain characters of the male terminalia and third larva, which suggest relationships with *Musca.* The species of the *calcitrans* group, on the other hand, would appear to be distantly related to *Musca.* I have, therefore, decided, in the meantime, to arrange the groups in the order given above, leaving the final decision as to which group contains the simplest species for discussion on another occasion, when I have studied the male terminalia of more species and especially those of allied genera.

It will be noted that the *stimulans* group contains species which exhibit greater variation in external structure than do the species of the other groups ; it contains, for instance, the following :—

1. Species in which the arista has hairs on both sides, and in which the palps are long and dilated in various ways.
2. At least two species in which the arista has hairs on both sides, though the palps are of uniform diameter throughout.
3. At least three species in which the arista has hairs only on one side, and in which the palps are long and dilated (not club-like).
4. Species which exhibit considerable variation in the arrangement and number of hairs and bristles on the body.
5. Species which have hairs on vein R_{4+5}.
6. Species in which vein R_{4+5} is without hairs.

The three species of the *irritans* group, though all agreeing in having long dilated palps and hairs on only one side of the arista, differ in certain other characters, such as those of the anal vein and the structure of the hind tarsi of the male. The species of the *maculosa* and *calcitrans* groups, on the other hand, exhibit great uniformity in structure, the species of each being very alike. In both groups, the arista has hairs only on the upper surface, but this common character does not indicate any very close relationship within the genus. In the *maculosa* group the species have dilated palps and a short stout proboscis, whereas in the *calcitrans* group the palps are short and of uniform diameter, and the proboscis is always long and slender.

CLASSIFICATION OF STOMOXYS (sens. lat.) ACCORDING TO MR. MALLOCH.

Many attempts have been made to classify the species of *Stomoxys* into smaller groups by the use of various external characters. No useful purpose would, however, be served by attempting to give here a historical account of these classifications, especially as I am not concerned with vexed questions of nomenclature, but only with the truth regarding the identities of the species, their relationships to each other, and the easiest and most certain means of identifying them. I cannot, however, pass over without comment the latest and most pretentious of these, recently given by Mr. Malloch.

In a series of three papers, Mr. Malloch (1932) has given a classification of *Stomoxys* (sens. lat.) with keys and short descriptions of some 48 species. He raises the genus to the rank of a subfamily, the Stomoxydinae, and divides it into two tribes, the Stomoxydini and the Haematobiini, and recognises nine genera and five subgenera, some of which are new ; the tribes are defined as follows :—

Tribe Stomoxydini. ' Contains species in which the palpi are entirely cylindrical, all those known to me having them undilated at apices, with the inner surface of each almost as the outer, though not so distinctly haired or almost without hairs.'

Tribe Haematobiini. ' Distinguishable from Stomoxydini by the structure of the palpi, these being so constructed, with the inner side grooved, shining, and generally bare, that they can be pressed closely against the sides of the proboscis much as in the Glossinidae.' He includes the following genera in the two tribes :—

Tribe Stomoxydini
Genus *Stomoxys*
„ *Haematostoma*
„ *Bdellia*
„ *Neivamyia*
„ *Bruceomyia*

Tribe Haematobiini
Genus *Stygeromyia*
„ *Bdellolarynx*
subgenus *Lyperosiops*
„ *Bdellolarynx*
„ *Haematobina*
Genus *Haematobosca*
„ *Haematobia*
subgenus *Haematobia*
„ *Glossinella*

In referring to the use of the characters of the palps for the classification of these flies, he says:—' When I described the species [*B. flavicornis*] I was not inclined to consider the structure of the palpi as of particular significance as a criterion for the separation of genera, dilated and undilated palpi appearing to me as described by the various authors who have dealt with these insects as of no more importance here than elsewhere in the Muscidae. However, with access to a much larger amount of material, including some genera previously unknown to me, I have changed my opinion on this matter and now believe that the palpi do indicate in certain details some possible lines of relationships. It is not so much the mere widening of these organs at their apices that I refer to as indicating phylogenetic relationships, as the nature of the inner surfaces. I have utilised this character in my key to the genera presented upon a preceding page in this paper, and in pursuing the scheme outlined therein and slightly more elaborated under the tribe heading on a previous page I have placed in the Stomoxydinae [Stomoxydini] several genera including *Neivamyia*. This action has rather widely separated the present genus [*Neivamyia*] from *Bdellolarynx*, in which I originally placed *flavicornis*, but I am convinced that this is the proper course to follow.' In the key referred to, the two tribes are distinguished as follows :—

Tribe Stomoxydini. ' Palpi cylindrical, slender, not appreciably thickened at apices, and with the inner side regularly rounded and not shining.'

Tribe Haematobiini. ' Palpi not cylindrical, generally more or less dilated apically, always thickened from a little beyond base to apex, the inner side of each palpus distinctly grooved, or hollowed out, and generally distinctly shining or polished.'

As the grouping of the species I have given above is directly opposed to that of Mr. Malloch, it is necessary to examine critically the characters on which his classification is based. I have studied microscopically the palps of many typical examples of Mr. Malloch's tribes, and will now shortly describe and illustrate a few. The specimens from which the drawings were made were dissected off

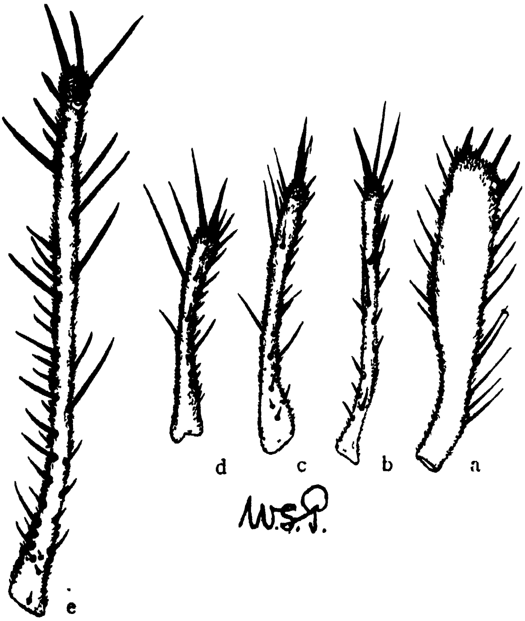

FIG. 13. *a.*—Inner surface of right palp of female *exigua* ; *b.*—Same of left palp of male *punctigera* ; *c.*—Same of right palp of male *calcitrans* ; *d.*—Another of same of male *calcitrans* ; *e.*—Same of left palp of male *lutzi*. All drawn to same scale.

the proboscis, after clearing the head in caustic potash, dehydrating, etc., and were then mounted on slides, and drawn to the same scale.

TRIBE STOMOXYDINI. Two right palps of the male *calcitrans* are illustrated in fig. 13, *c*, *d*; the inner surface, that nearest the proboscis, is facing the reader. In both it will be noted that the palp is about the same width throughout, though if anything slightly wider at the ends, and also that it may vary in length; it is always much shorter than the proboscis, and in the position of rest lies against its side. It is a chitinous tube enclosing a space, the haemocoele. Under a high magnification it will be noted that it is covered all over with minute pointed spinulae, and the outer surface, and to a slight extent the inner, which is narrow, bears many bristles. In fig. 13, *b*, the left palp of the male *punctigera* is illustrated, and it will be noted that it is about as long as that of *calcitrans*, is structurally similar but narrower, and is armed with spinulae and bristles. In fig. 13, *e*, the left palp of the male *lutzi* (*Neivamyia*) is illustrated; though much longer, it is also structurally similar, and has spinulae and bristles. In describing this type of palp, Mr. Malloch refers to the inner surface as being ‘ almost as the outer though not so distinctly haired, or almost without hairs ’; presumably the hairs referred to are the bristles. The inner surface is, however, covered with fixed hairs as is the outer, but has only a few bristles.

TRIBE HAEMATOBIINI. The right palp of the female *S.* (*Bdellolarynx*) *sanguinolentus* is illustrated in fig. 14, *d*, and, as in the case of the others illustrated, the inner surface (that applied to the proboscis) is facing the reader. It will be noted that this palp is gradually dilated from about its middle. The outer surface is more strongly chitinised than the inner which is more membraneous, and both are covered with the same minute pointed spinulae; the outer surface and edges have, in addition, many strong bristles. The inner membraneous wall in life is *flat* or even *convex*, the space between the two walls is the haemocoele, and is filled with blood, nerves, tracheae, etc. This palp, therefore, cannot be referred to as grooved or hollowed out. In fig. 14, *c*, *b*, are illustrated in each case the right palp of the male and female *S.* (*Lyperosiops*) *stimulans* respectively. This palp, it will be noted, is much more definitely club-shaped, and there is not much difference in its structure in the sexes. It is structurally similar to that of *sanguinolentus*. In fig. 14, *a*, is illustrated

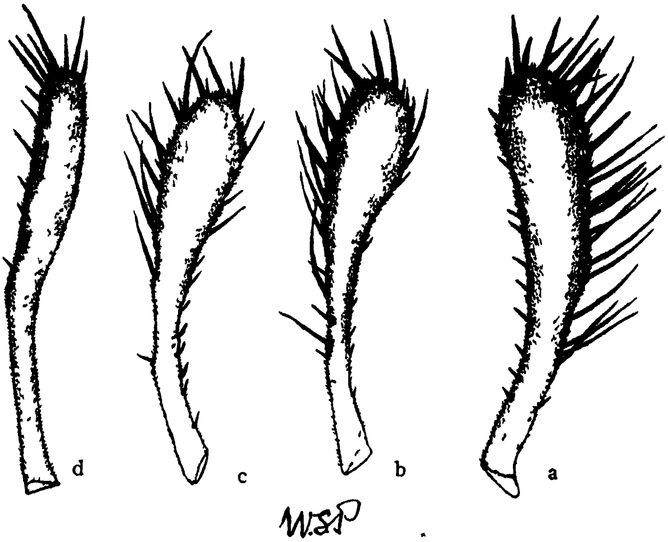

FIG. 14. *a*.—Inner surface of left palp of female *maculosa* ; *b*.—Same of right palp of male *stimulans* ; *c*.—Same of female of *stimulans* ; *d*.—Same of right palp of female *sanguinolentus*. In each case the inner surface (facing) is convex and not hollowed out ; all drawn to same scale.

the left palp of the female *S.* (*Stygeromyia*) *maculosa* ; it is much more club-shaped, the dilatation commencing nearer the base than in the case of the palp of *stimulans*. It is also structurally similar to the others. The inner surface of these palps, owing to the width and membraneous nature, has a somewhat light appearance ; it is not bare, however, as is suggested by the term ' shining.' Lastly, the palp of the female *S.* (*Haematobia*) *exigua* is illustrated in fig. 13, *a* ; this palp is expanded, and the surfaces are structurally similar to those of the others noted, but the bristles are shorter.

Mr. Malloch describes this type of palp as being ' so constructed with the inner side grooved, shining and generally bare that they can be pressed closely against the sides of the proboscis much as in the Glossinidae.' It will be noted, however, from the above short descriptions and from the illustrations, that this type of palp is not grooved, that its inner surface, though not usually armed with bristles, is covered with spinulae. Further, these palps are never pressed closely against the *sides* of the proboscis when at rest. In order to demonstrate this I have mounted the heads of several species, with the proboscis and palps in the position of rest, and one of these

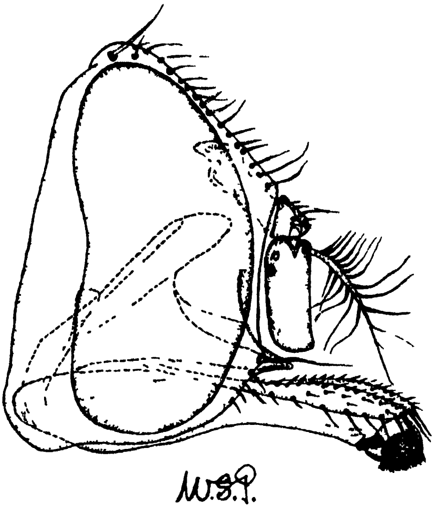

FIG. 15. Head of female *chinensis* in side view, to show resting position of proboscis ; note how the two palps lie on the dorsal surface, the bristles at the end protecting the labella.

is illustrated in fig. 15. It will be noted that the palps are applied, not to the sides, but to the *upper* surface of the proboscis, so that the strong terminal bristles come to lie over the labella. This is a special provision to protect from possible injury the most important part of the proboscis, the labella, which bear the tearing and piercing armature. In *Glossina* the palps *completely* enclose the proboscis. It will be noted then that Mr. Malloch has misinterpreted the structure[1] of the palp in *Stomoxys* (sens. lat.), and especially that part on which he bases the primary divisions of his classification ; for he says : ' It is not so much the mere widening of these organs at their apices that I refer to as indicating phylogenetic relationships, as the nature of the inner surfaces.' I can find no such differences in the inner surfaces of typical examples of the palps of the two groups. Except for the differences in width, the inner surface is similar in both, and that of the dilated palp is not grooved or hollowed out, but flat or even convex. Using the characters of the palp, Mr. Malloch groups all those species in which they are of uniform diameter and in which the inner surface is like the outer, into the tribe Stomoxydini, and those in which they are dilated, the inner surface (erroneously described as) grooved and shining, into the tribe Haematobiini.

In the tribe Stomoxydini are included five genera, the species of two of which, *Haematostoma* and *Bdellia*, are unknown to me. I have, however, studied the male terminalia of species of the remaining three genera, *Stomoxys* (sens. rest.), *Neivamyia* and *Bruceomyia*. The species of *Stomoxys* (sens. rest.), as noted above, fall into my *calcitrans* group, which probably represents the most advanced species of the genus. *N. lutzi*, which Mr. Malloch says is identical with his *N. flavicronis*, and *B. punctigera* are both closely related to each other and belong to my *stimulans* group, the species of which are distinct from *calcitrans* and its allies (*Stomoxys*, sens. rest.) ; the species of this group, it will be remembered, exhibit great variation in external characters, and at present I consider that they are the simplest. The undilated palps of *lutzi* and *punctigera* have, therefore, misled Mr. Malloch into considering that these two species are related to *calcitrans*. I, therefore, consider that there is no necessity for the genera *Neivamyia* and *Bruceomyia*--certainly not for the latter, which is based on the slenderest possible characters. It will be remembered that *punctigera* has in the past been placed in the genus *Lyperosia* (now *Haematobia*, according to Mr. Malloch), and thus associated with such species as *irritans*, *minuta* and *exigua*, which are quite distinct and belong to my *irritans* group.

In the tribe Haematobiini, as noted above, Mr. Malloch includes four genera and five subgenera. With the exception of the subgenus *Haematobina*, I have examined the male terminalia of many species of all the genera and subgenera. The species of the genus *Stygeromyia* (*maculosa* group) are like those of the *calcitrans* group (*Stomoxys*, sens. rest.) in being of a uniform structure, and, therefore, form a natural group, nearest, I believe, to the species of the *calcitrans* group. Mr. Malloch, on the other hand, has been misled by the structure of the palp in this group, and places the species in his tribe Haematobiini, thus allying them to the species of *Haematobia*, etc. ; he appears to have examined only one specimen of *Stygeromyia*. In the definitions of his remaining genera and subgenera of the Haematobiini, and from the species he associates together in them, it is quite clear that he really does not understand either the significance of the characters he uses or the true relationships of the species. For instance, he places *stimulans* and *sanguisugens* in the subgenus *Lyperosiops*, and *sanguinolentus* and *uniseriatus* in the subgenus

Bdellolarynx. On the characters of the male terminalia all four are closely related to each other and belong to the *stimulans* group, and in this group *uniseriatus* is more closely related to *perturbans* than to either *sanguinolentus* or *squalida* with which Mr. Malloch associates it. Again, Mr. Malloch groups *thirouxi* and *titillans* in the subgenus *Glossinella*, allying them with *minuta*, *potans* and other species ; *minuta* is, however, related to *irritans* and *exigua* in the *irritans* group, whereas *thirouxi* and *titillans* belong to the *stimulans* group.

I have said enough to show that Mr. Malloch's classification is not only most unsatisfactory but is inaccurate and misleading, for it is based on a fundamental error regarding the structure of the palps, which organs unfortunately do not indicate any ' possible lines of relationships,' which Mr. Malloch considers they do. Further, Mr. Malloch has made new species and erected new genera on the slenderest possible grounds, two of his species are each based on a single specimen, and one genus on a single specimen, and that a *greasy* female. No types of the older authors appear to have been examined, and no larvae studied. I very much regret to have to criticise again unfavourably Mr. Malloch's systematic studies on the higher Diptera, but I feel that the time has now come for plain speaking on this subject. I am more than ever convinced that no advance, but rather the reverse, will be made in our knowledge of the status, affinities and relationships of these insects along the lines of Mr. Malloch's studies. The comparative study of the male terminalia offers the only sure means of clearing up the many puzzling problems connected with the higher Diptera. I would, however, again like to emphasize what are the primary objects of these comparative studies—viz. : (1) to fix the identities of the species, especially in the case of closely related ones ; (2) to discover the true relationships of species within a genus, and their relationships to those of allied genera. With this accurate information it will be possible to build up a natural classification. I would, therefore, like to draw attention to the note of warning given elsewhere (1933) on the danger of drawing conclusions as to the identities and relationships of species and genera from incomplete studies of the male terminalia ; such studies have in the past tended to discredit the value of the terminalic characters. It should be remembered that the characters of the male terminalia are not those of a single organ but of several embryonic segments. In only a very few genera have the female terminalia been studied from the comparative standpoint. In two genera at least *Musca* (Awati) and *Glossina* (Evans), the parts exhibit distinct correlation with those of the male, and are even useful in identification. The fact of the matter is that the female terminalia are even more difficult to study than those of the male, and few have cared to explore this field ; that is, however, no proof that the female does not possess good terminalic characters (see another paper in this journal).

With regard to the making of new species and the erecting of new genera, I have now for many years adhered to the following two rules, and have refrained from making new species and new genera without first complying with them. They are as follows :— RULE I. No new species should be made without first (*a*) examining the type, cotype, etc., of a species closely allied to the new one, (*b*) examining the male terminalia of the new species, and comparing them with those of allied forms. RULE II. No new genus should be erected without first examining the male terminalia of the type species and comparing them with those of species of allied genera.

As a result of my comparative studies of the male terminalia of *Stomoxys* (sens. lat.), I can see no justifiable reason for dividing the genus into smaller genera, especially now that it is quite clear that the external characters used for defining these genera has resulted in the grouping together of unrelated species, and separating closely related forms in distinct genera. All the species are closely related, the male terminalia and third larva exhibiting fundamental generic characters, such as the uniform structure of the phallosome, the absence of its posterior process, the presence of the horned sclerite, the fused anal cerci, the very similar proximal segment of the ninth coxite, the cephalopharyngeal skeleton of the larva with its very small left oral hook, and the structure of the posterior spiracles ; the female terminalia also exhibit generic characters. The splitting of this natural group into smaller genera for the reason that it has become unwieldy (there are only about 50 species known) is, in my opinion, an unscientific procedure to say the least of it, and it has not produced the object which Mr. Malloch attaches to it, which, to quote his words, is ' so that a closer association of related forms in different faunal regions may be thus more definitely indicated.' It has on the contrary not only led to much confusion and a misleading classification, but has rendered identification a tedious and difficult task. I have found it well nigh impossible, even with the special knowledge I possess, to say whether a particular species is a *Haematobia*, *Bdellolarynx*, *Nievamyia*, *Haematobosca*, etc. And I am quite sure that veterinary officers, to whom these flies are of the utmost importance, would find it absolutely impossible to identify any of these genera. I have, therefore, decided to sink them and to place all the species in the single genus *Stomoxys*, and to arrange them in the four groups, as noted above. This change will greatly simplify identification, for it will only be necessary to recognize the fly as being a *Stomoxys*, and that is a simple matter, as all the species possess two characters in common, viz. : a forwardly directed, non-retractile proboscis, which at rest lies in a horizontal position projecting forwards to varying degrees in front of the head; and vein M_{1+2} bends forwards with a rounded bend to a less or greater extent towards vein R_{4+5}. It will then be an easy matter to identify the species with separate keys to those of the different regions. A single key to the species of more than one region is very confusing to use, and, besides, the worker in Africa does not want to identify any Oriental or Chinese species. The following is a revised definition of the genus *Stomoxys*.

Genus Stomoxys Geoffroy (emended Patton, 1933)

Generic Characters. Larva. Third stage larva with attenuated anterior end, long cephalopharyngeal skeleton and very small left oral hook, much smaller than that of the larva of *Musca* ; posterior spiracular plates D-shaped, tending to become round, either widely separated or close together ; the three sinuous breathing slits long and narrow, and the button central. Adult. Arista with spinulae either on both sides or only on the upper surface ; palp either short, and then much shorter than the proboscis, or as long as the proboscis, and either of uniform diameter throughout or dilated, and then always lying on the upper surface of the proboscis ; proboscis non-retractile, and projecting horizontally forwards from under the head, either short and stout, or long and markedly narrow, or very long and slender ; labium sometimes bulbous at base ; labella much reduced, and armed with strong teeth and an interdental armature. Sternopleural bristles variable, either 0 : 1, 1 : 1 or 1 : 2. Vein M_{1+2} bends forwards towards vein R_{4+5} with a gentle, rounded bend, ending either some distance from or very close to it (*maculosa* group), so that cell R_5 may have a bottle-neck opening. Male Terminalia. Anal Cerci. Broad, triangular (wing-shaped), fused in the middle line to a varying extent so that the inner ends of the free margin may project like nipples to some extent. Ninth Coxite. Proximal segment elongated, distal end either narrowly or broadly rounded ; anterior border with or without a process of varying length ; distal segment concealed, a narrow hairy plate lying in between membrane at outer margin of anal cercus. Ninth Tergo-Sternum. Y- or V-shaped, strongly chitinised, postero-lateral processes long, strongly chitinised, and bending round posteriorly towards each other. Phallosome. Uniform in structure throughout the genus, consisting of two chitinous tubes, the distal long and funnel-shaped, and always with a characteristic horned sclerite at distal end, placed at right angles to long diameter of phallosome ; it may be bicornuate. POSTERIOR PROCESS OF PHALLOSOME. Always wanting. PUMP SCLERITE OF EJACULATORY DUCT. Small. PARAMERE. Consists of two *distinct* parts joined by membrane ; anterior portion, a raised process of very similar structure throughout the genus, and armed with 1-3 bristles ; posterior part, a narrower raised process, bent forwards, either with or without hairs at distal end, its structure varying to a small extent.

The following is a complete list of the species known to me at present. An asterisk accompanying a name means that I have examined the type, and a query that I am doubtful as to whether the species belongs to the group in which it is placed.

GENUS *Stomoxy* GEOFFROY

Stimulans Group

?**angustifrons* Malloch (?*uniseriatus* Malloch). Zululand.
?**atripalpis* Bezzi. Italy.
**chinensis* sp.nov. North China.
**equina* Enderlein. Southern Hungary.
? *flavicornis* Malloch (?*lutzi* Pinto & Fonseca). Brazil ; Surinam.
?**hirtifrons* Malloch. Kenya.
? *hirudo* Enderlein. Cameroons.
? *latifrons* Malloch. Peru ; Brazil.
? *latirostris* Enderlein. Corsica.
lutzi Pinto & Fonseca. Brazil.
? *mallochi* Patton (*B. latifrons* Malloch). Nyasaland ; Zululand.
**perturbans* Bezzi. North China.
? *pallidipes* Roubaud. French and Belgian Congo.
? *potrix* Enderlein. Socotra.
? *praedatrix* Enderlein. Cameroons.
**sanguinolentus* Austen (*nudinervis* Stein ; *rufipes* Stein). India ; Middle and Far East.
**sanguisugens* Austen. North India.
?**shillingsi* Grünberg. East Africa.
**squalida* Grünberg (*lutosa* Grünberg, nom. nud.) Tanganyika.
?**spinigera* Malloch. Uganda ; Nyasaland.
stimulans Meigen (*alcis* Snow). Europe ; N. America.
thirouxi Roubaud. Senegal.
**titillans* Bezzi. Southern Europe.
**uniseriatus* Malloch. Southern Rhodesia ; Zululand ; Tanganyika.

Irritans Group

exigua de Meijere (**australis* Malloch ; **flavohirta* Brunetti). Oriental, Australasian and Palaearctic regions ; Oceania ; N. China ; Iraq.
irritans L. (*serrata* R-D.) Europe.
**minuta* Bezzi (*longipalpis* Roubaud). Somaliland ; Oriental and Ethiopian regions.
?**potans* Bezzi. Eritrea ; South Africa.

Maculosa Group

**maculosa* Austen (*rufipalpis* Becker). Oriental region ; Aden.
**pottsi* sp.nov. Nyasaland ; Tanganyika.
**sanguinaria* Austen. Katanga, Belgian Congo, Nyasaland ; Tanganyika.
**woosnami* Austen. Kenya.

Calcitrans Group

bengalensis* Picard (discalis* Malloch ; **oblongopunctata* Brunetti). India ; Malay ; Burma.
**bilineata* Grünberg. Tanganyika.
**boueti* Roubaud. Liberia ; Dahomey ; Tanganyika.
**bouveri* Roubaud. French and Belgian Congo ; Dahomey.
**brunnipes* Grünberg (*sellata* Grünberg). Cameroons ; Tanganyika ; French and Belgian Congo ; Rhodesia ; Nyasaland.

Calcitrans Group (*continued*).

calcitrans Linnaeus (*plurinotata* Bigot). Cosmopolitan.

**dubitalis* Malloch. South India.

indica* Picard (limbata* Austen; **pratti* Summers; **pusilla* Austen). Oriental region; N. China.

nigra* Macquart (glauca* Grünberg; *lafonti* Picard). Island of Rèunion; Mauritius; widely distributed in Africa.

ochrosoma* Speiser (flavida* Malloch). Tanganyika.

**omega* Newstead. Widely distributed in W. Africa.

**inornata* Grünberg. West Africa.

pallida* Roubaud (neivei* Malloch). Dahomey; Nyasaland.

**pulla* Austen. Mussoorie, N. India.

rhodanica Roubaud. Uganda; Belgian Congo.

sexvittata Roubaud. French Sudan.

**sitiens* Rondani. Abyssinia (Bogos).

**taeniata* Bigot. Natal; Katanga, Belgian Congo; Kenya; Tanganika; Nyasaland.

**transvittata* Villeneuve. Natal.

**triangularis* Brunetti. South India.

**varipes* Bezzi. East Africa; Abyssinia.

It will be noted from the above list that I have examined either the type, cotype or a conspecific specimen of all but some eight species, and have, therefore, cleared up a large amount of synonymy. I have not yet had an opportunity of examining the male terminalia of any of the species of *Haematobina* Malloch. In the list, under the *stimulans* group, I have included many species which I believe in all probability belong here; this can, however, only be settled when I have had an opportunity of examining their male terminalia. I have not yet been able to examine the male terminalia of *potans* Bezzi and have only provisionally placed it in the *irritans* group. After examining specimens named by Summers from Kuala Lumpur and the large collection I have had from the same locality, I have been able to settle the identity of Malloch's *discalis* and Brunetti's *oblongopunctata*; and *pratti* is identical with *indica*. I have seen neither the true *nigra* nor *brunnipes* from any part of the Oriental region. Bigot's *plurinotata* is only *calcitrans*. After examining specimens of *sitiens* Rondani, *bilineata* Grünberg, *brunnipes* Grünberg and *varipes* Bezzi determined by various authors, it is quite clear that there is much confusion as to their identities. It would only be guess-work to attempt to settle the identities of these species without further study of the types. As I have sunk all generic names as synonyms, it becomes necessary to rename Malloch's *Bdellolarynx latifrons*, as this name is preoccupied by *Neivamyia latifrons* Malloch, and I have much pleasure in re-naming it *Stomoxys mallochi*.

The revision of the species, together with regional keys and black and white illustrations of all the known species, will be published in due course in Part 3 of my book, ' Insects, Ticks, Mites, etc.' I would like to take this opportunity again of appealing to medical and veterinary officers and medical entomologists to let me have any of the rarer species, especially those found on game animals ; a list of these will be found in my two previous papers, copies of which will be sent to anyone wishing for them. I shall be glad to name any collections of *Stomoxys*. In my next paper in this series I shall deal with the genus *Glossina*. I have now studied the male terminalia of all the known species, and shall attempt to explain their relationships to each other and discuss the systematic position of the genus.

SUMMARY

1. The comparative study of the male terminalia of 31 species of *Stomoxys* (sens. lat.) has proved the parts to be of a uniform structure, that of the phallosome, the absence of its posterior process, the presence of the horned sclerite, the fusion and uniform structure of the anal cerci, and that of the proximal segment of the ninth coxite, all demonstrate the close affinities of the species ; the larval characters, too, confirm these findings. There is, therefore, no valid reason for splitting the genus into smaller genera.

2. The species fall into four natural groups on the characters of the male terminalia—viz. : the *stimulans*, *irritans*, *maculosa* and *calcitrans* groups—and belong to the single genus *Stomoxys* ; their exact position within the genus is, however, not yet quite clear.

3. The *stimulans* group is a remarkable one, for it contains species with long and short undilated palps, others in which they are dilated, species in which the arista has hairs on both sides, and others again in which the under surface is bare. The *irritans* group also contains species which have some variable external characters. The *maculosa* and *calcitrans* groups, on the other hand, contain species which all possess a uniform set of external characters.

4. The presence or absence of hairs and bristles, the structure of the palp, that of the antenna, that of the male hind tarsus, and the length of the anal vein, characters which have largely been used for grouping the species, have proved to be, as the result of this study, misleading guides to the true relationships of the species.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

COCHLIOMYIA AMERICANA SP.NOV., THE SCREW-WORM FLY OF THE NEW WORLD

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Recently, while undertaking a comparative study of the male terminalia of species of the genera of the Calliphorinae, we had occasion to examine those of two collections of what we believed were adults of *Cochliomyia macellaria* F., which one of us (E. C. C.) had collected earlier in the year in Texas. The history of these two collections is as follows :—

COLLECTION A. This collection consists of adults taken at random from a large number caught in a range blow-fly trap at Menard, Texas, during the latter part of July, 1933.

COLLECTION B. This collection consists of adults reared from larvae which had matured in wounds in experimental cattle at Menard, in July, 1933. The animals were wounded, and the wounds were allowed to become infested in nature. The mature larvae, on leaving the tissues of the several animals, migrated into a special receptacle containing sand, and were allowed to pupate. Adults emerging from the puparia were selected at random and form this collection.

On comparing microscopical mounts of the male terminalia of these two Calliphorinae (A) and (B), one of us (E. C. C.) discovered that we were dealing with two distinct species. A comparison of the male terminalia of species of collection A with those of others of the typical *macellaria* in our possession leaves no doubt that they are identical, and that the trapped adults (collection A) are the true *macellaria*, whereas the adults of collection B are abundantly distinct and represent an undescribed species, which we propose naming *Cochliomyia americana.* We give below a description of the adults of this new species and of the male and female terminalia, comparing them with those of *C. macellaria.*

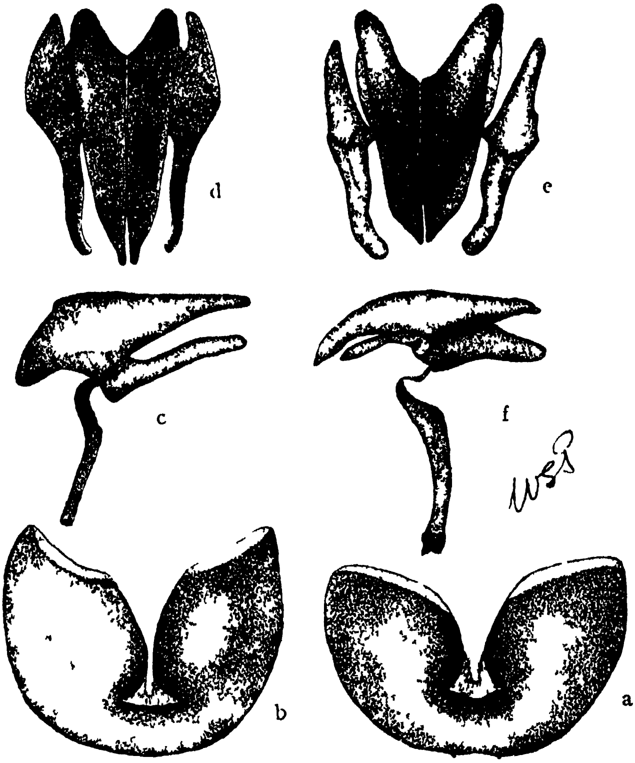

FIG. 1. *a.*—Fifth sternum of ♂ *americana* ; *b*—Same of ♂ *macellaria* ; *c*—Lateral view of anal cerci and two parts of ninth coxite of *macellaria* ; *d*—Ventral view of same of *macellaria* , *e.*—Ventral view of same of *americana* , *f.*—Lateral view of same of *americana* (all to same scale)

Cochliomyia americana sp.nov. A dark bluish-green fly with white dusting and three broad, dark, mesonotal stripes ; the abdomen unbanded. Average length of 10 males, 9 mm., of 10 females, 8·8 mm.

MALE. HEAD. Vertex greyish-yellow, very narrow (linear), about one-seventh width of an eye ; vertical stripe linear, chocolate in colour ; cheeks orange, with golden or silky hairs ; occiput with similar hairs. *Antennae.* Scape, dark orange to black ; pedicel, dark orange with reddish tinge along lower border ; anterior border of first segment of flagellum, dark grey, and in some lights whole segment appears grey ;

arista, dark orange to black, with black spinulae on both sides almost to tip. *Palps*. Orange, small and of uniform diameter (they vary in length). THORAX. *Mesonotum*. Dark bluish-green, anterior half with marked white dusting and three broad dark stripes extending almost to scutellum, one median and one on each side ; a pair of much narrower dark prescutal stripes, slightly divergent, one on each side of median dark stripe ; dorsocentral and acrostichal bristles much reduced and represented only by a pair of each at posterior end of mesonotum, which is covered with numerous fine dark hairs. WING. Costal cell on each side of humeral cross vein (especially outer side) pigmented ; cells Sc, R_1 and R also pigmented at the basal ends (the amount varying in different specimens). ABDOMEN. Uniformly dark green with white dusting on terga 3 and 4 ; a narrow dark triangular median stripe on tergum three.

MALE TERMINALIA. *Anal Cerci*. Ventral view. Fig. 1, *e*. Short, about the same width throughout, and narrowing abruptly to pointed ends ; side view as illustrated in fig. 1, *f*. *Ninth Coxite*. *Distal Segment*. Ventral view. Fig. 1, *e*. Narrow and rounded, of uniform width ending in rounded points ; lateral view as in fig. 1, *f*. *Proximal Segment*. Lateral view. Fig. 1, *f*. A strong twisted rod united to distal segment by a tendon. *Ninth Tergo-Sternum*. Fig. 2, *g*. Short, broad, without definite shaft. *Phallosome*. Lateral view. Fig. 2, *a*. Distal part of body arched, not elongated as in *macellaria* (fig. 3, *a*), and bent almost at a right angle to the apodeme ; forking of proximal end of body not so deep as in *macellaria* (fig. 3, *a*), so that the anterior end does not form such a prominent process. *Posterior Process of Phallosome*. Fig. 2, *a*. Short, straight, tapering to a point and not bent as is that of *macellaria* (fig. 3, *a*). *Apodeme of Phallosome*. Fig. 1, *a*. Shorter and stouter than that of *macellaria* (fig. 3, *a*). *Sperm Pump Sclerite*. Fig. 2, *c*. Fan-shaped, handle relatively long (much longer than that of *macellaria*, fig. 3, *b*), and body narrower. *Parameres*. Lateral view. *Anterior Part*. Fig. 2, *a*, *b*, *f*. A short, rather deep, folded plate, anterior end rounded and slightly bent over, armed with 8-12 bristles, some smaller than others, and with short accessory process. *Posterior Part*. Fig. 2, *a*, *d*, *e*, *h*. A broad, upstanding process, distal end narrow, twisted and bent forwards, but not hooked as is that of *macellaria* (fig. 3, *a*, *d*, *f*, *g*). *Fifth Sternum*. Fig. 1, *a*. Broad, lateral lobes distinctly shorter and broader than those of *macellaria* (fig. 1, *b*), the notch broader and shallower.

FEMALE. HEAD. Vertex greyish-yellow, wide, nearly half the width of an eye ; vertical stripe chocolate, with either straight or slightly emarginate sides, and about half width of vertex, with 10-14 vertical bristles ; outer vertical bristles (parafrontal, orbital) wanting, but with hairs in several rows ; cheeks as in male. *Antennae*. First flagellar segment usually lighter than that of male, especially near base. *Palps*. As in male, but usually slightly longer. THORAX. Mesonotum as in male. WING. Pigmentation as in male. ABDOMEN. As in male, but usually more silvery dusting, and terga 1 and 2 darker.

FEMALE TERMINALIA. *Ovipositor*. (Segments 6, 7, 9 and 10.) Fig. 4. Rather short, about one-third shorter than that of *macellaria* (fig. 6). *Sixth Tergum*. Fig. 4, *m*. A wide arched plate, narrower than that of *macellaria* (fig. 4, *l*), bearing at outer edges the sixth and seventh spiracles, the latter smaller and well separated from the former. *Sixth Sternum*. Fig. 4, *n*. Elongated and expanded distally. *Seventh Tergum*. Fig. 4, *o*. Two wide plates usually attached distally, separated, and diverging proximally. *Seventh Sternum*. Fig. 4, *p*. Long and wide. *Ninth Tergum*. Fig. 4, *r*. Two distinct, rather short wide plates commonly united distally, separated proximally. *Ninth Sternum*. Fig. 4, *q*. A small, quadrilateral, lightly chitinised plate lying below the genital opening. *Tenth Tergum*. Fig. 4, *s*. A short cone-shaped plate

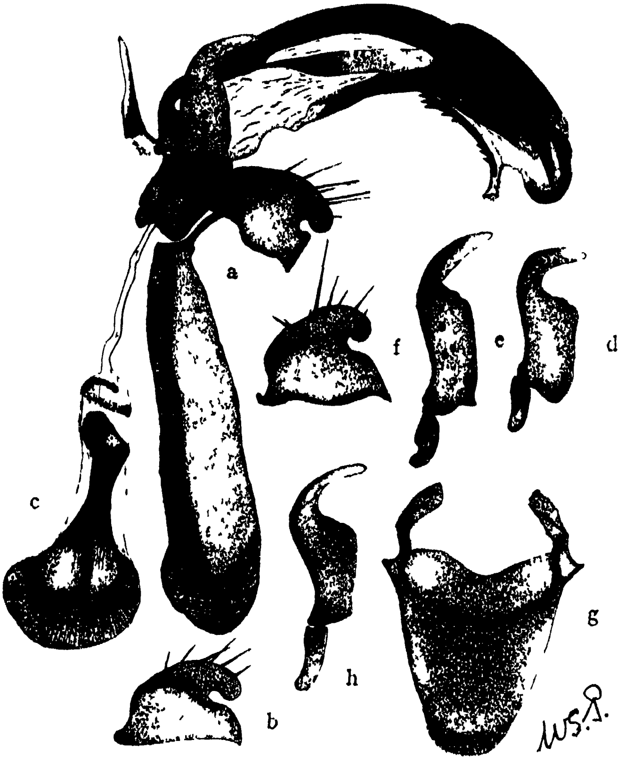

FIG. 2. *a.*—Lateral view of phallosome and one paramere of *americana* ; *b.*—Lateral view of anterior part of left paramere of same ; *c.*—Pump sclerite of same ; *d.*—Lateral view of posterior part of left paramere of same ; *e.*—Lateral view of posterior part of right paramere of same ; *f.*—Lateral view of anterior part of right paramere of same ; *g.*—Ninth tergo-sternum of same ; *h.*—Lateral view of posterior part of right paramere of same (drawings of parts same scale as corresponding ones of *macellaria*, fig. 3).

lying between anal cerci. *Tenth Sternum.* Fig. 4, *t.* A small wider cone-shaped plate ventral to anal opening, with a pair of strong rod-shaped sclerites supporting wall of genital opening. *Anal Cerci.* Fig. 4, *s.* A pair of elongated, club-shaped plates. *Spermathecae.* Fig. 4, *a.* Large, ovoid, strongly chitinised capsules with definite distal point ; ducts long.

The above description of the adults is based on the study of 150 males and 95 females ; many microscopical specimens of the male and female terminalia were also examined. We have carefully compared adults with those of *macellaria*, and are still unable to give any reliable external characters (except those of the terminalia noted below) which would enable a worker in the field to distinguish the two species. The adults of *americana* are, on the whole, larger and

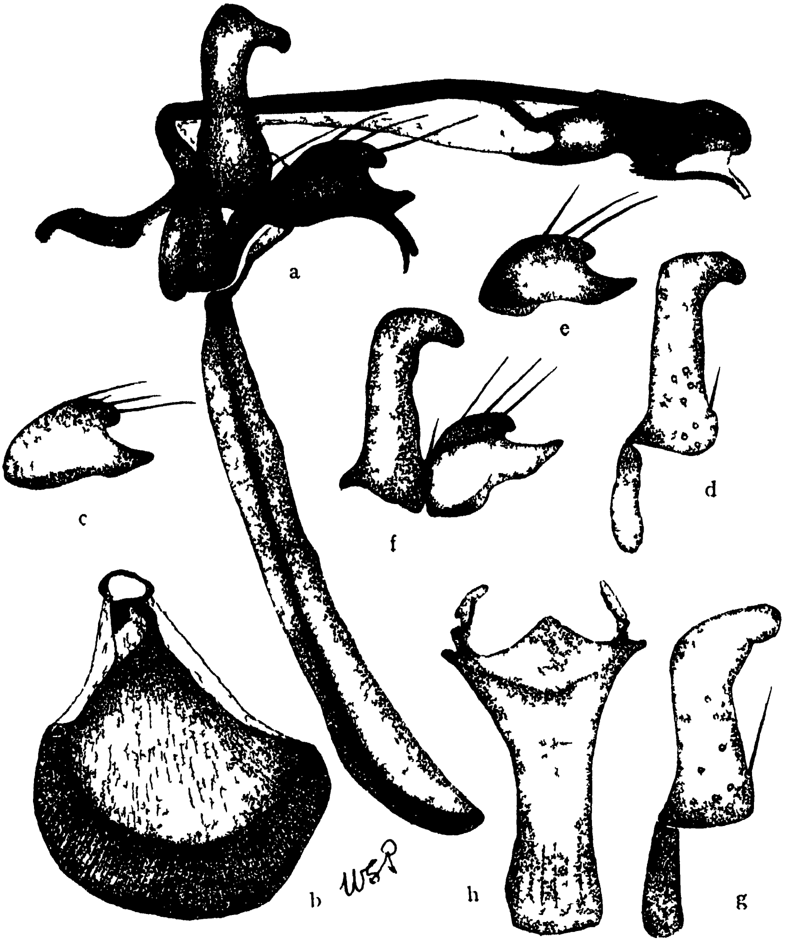

FIG 3 *a*—Lateral view of phallosome and one paramere of *macellaria* , *b*—Pump sclerite of same *c*—Lateral view of anterior part of right paramere of same , *d*—Lateral view of posterior part of right paramere of same , *e*—Lateral view of anterior part of right paramere of same , *f*— Lateral view of two parts of left paramere of same , *g*—Lateral view of posterior part of right paramere of same , *h*—Ninth tergo-sternum of same (cf fig 2, *g*)

of a darker colour than those of *macellaria*, but we do not attach much importance to these characters The fifth sternum of the male *macellaria* is appreciably different from that of *americana*, the lateral lobes being longer and more pointed, and the incision narrower and longer (fig 1, *a*, *b*) It requires only a little practice to appreciate these differences The female terminalia are strikingly distinct. The ovipositor of *macellaria* (fig 6, *b*) is about one-third longer than

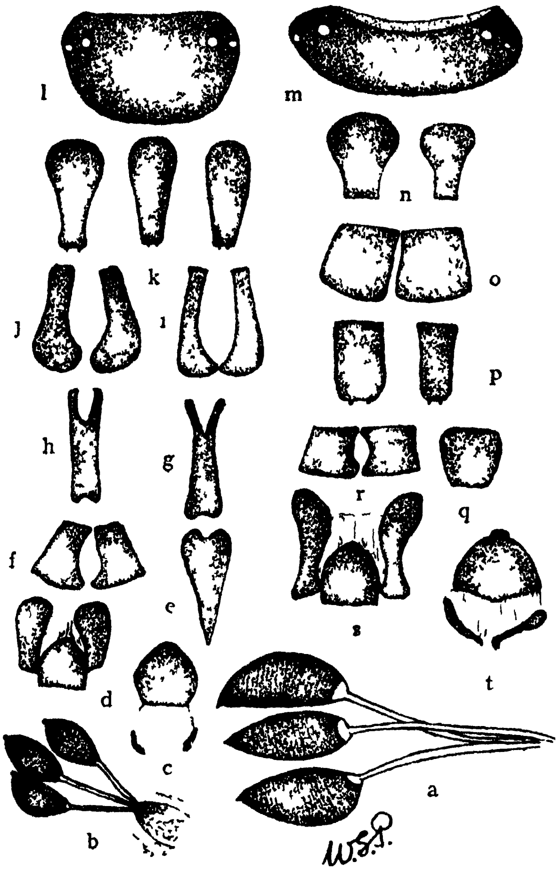

FIG. 4. *a.*—Spermathecae and parts of ducts of *americana*; *b.*—Same of *macellaria*, showing ducts entire and signum , *c.*—Tenth sternum of same ; *d.*—Anal cerci and tenth tergum of same ; *e.*—Ninth sternum of same (cf. *q*) ; *f.*—Ninth tergum of same , *g.*, *h.*—Seventh sternum of same (cf. *p*) ; *i*, *j*.—Seventh tergum of same (cf. *o*) ; *k*—Sixth sternum of same ; *l.*—Sixth tergum of same ; *m.*—Sixth tergum of *americana* , *n.*—Sixth sternum of same ; *o.*—Seventh tergum of same ; *p.*—Seventh sternum of same ; *q.*—Ninth sternum of same ; *r.*—Ninth tergum of same ; *s.*—Anal cerci and tenth tergum of same ; *t.*—tenth sternum of same ; note strong rod-like sclerites (all to same scale).

that of *americana* (fig. 6, *a*). It is only necessary to compress the abdomen and extend the ovipositor to detect the difference.

The other structural details showing the difference in the sclerites of the ovipositor are illustrated in figs. 4, 5, which are drawn from caustic potash preparations, and it is unnecessary, therefore, to describe them. The most striking differences are to be noted in the

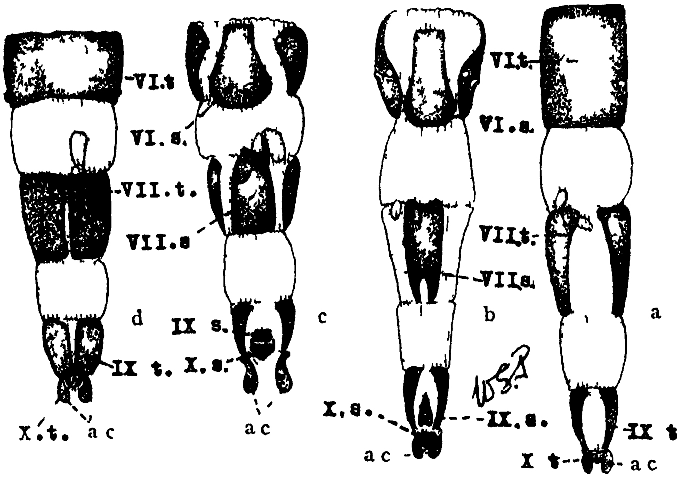

FIG 5 *a*—Dorsal view of ovipositor of *macellaria*, *b*—Ventral view of same, *c*—Ventral view of ovipositor of *americana*, *d*—Dorsal view of same, *VI t*, *VI s*—Sixth tergum and sternum, *VII t*, *VII s* Seventh tergum and sternum, *IX t*, *IX s*—Ninth tergum and sternum, *X t* *X s*—Tenth tergum and sternum, *a c*—anal cerci (to same scale)

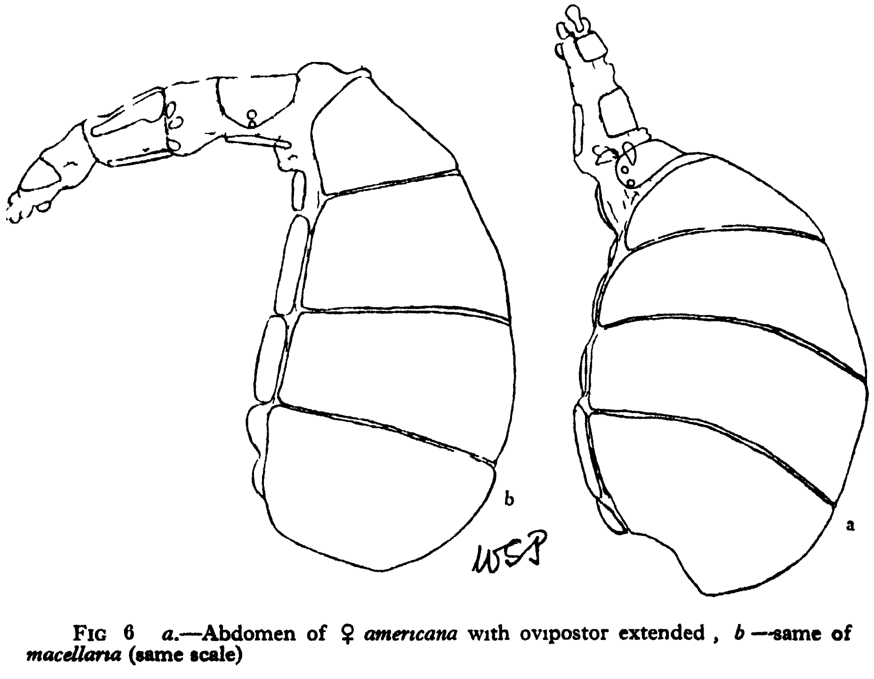

FIG 6 *a*.—Abdomen of ♀ *americana* with ovipostor extended, *b*—same of *macellaria* (same scale) sixth terga and sterna, the seventh terga and sterna, the ninth sterna and the anal cerci. The spermathecae of *macellaria* (fig. 4, *b*) are much smaller and are structurally different from those of *americana* (fig. 4, *a*), the ducts being very short. The differences in the male terminalia are striking and are brought out in detail in figs. 2, 3, which should be carefully compared. The long *straight* phallosome of *macellaria* is characteristic, as is also the much shorter *arched* one of *americana*. The structure of the former appears to be correlated with that of the ovipositor. We hope to discuss this on another occasion. We have also carefully compared the female *americana* with two females of *C. wheeleri* Hough, named by Dr. Aldrich and presented to this School, and note they are quite distinct. The palps of *wheeleri*, as first noted by Hough, are long and club-shaped, whereas those of *americana* are small and of uniform diameter ; the femora and tibiae of *wheeleri* are yellow, whereas those of *americana* are almost black. The female terminalia are also quite distinct and are nearer to those of *macellaria*. We are unable at present to describe and illustrate the male terminalia of *wheeleri*, as we have no specimens of this sex. In the meantime we accept the genus *Cochliomyia* which Townsend erected for these New World species of *Chrysomyia* ; we do not, however, accept his trivial generic characters ; we hope to discuss the status of this genus on another occasion.

In due course, the types of *americana* will be deposited in the U.S. National Museum, Washington ; paratypes in the Natural History Museum, London ; in the collection of the U.S. Bureau of Entomology, Washington ; in the Liverpool School of Tropical Medicine, Liverpool ; and in the collection of the London School of of Hygiene and Tropical Medicine.

Possible larva of C. americana. Although we have no larva of *americana*, none having been preserved at the time collection B was made, we have examined a larva from a case of myiasis from Mexico in the collection of this School. The larvae were collected by Dr. Hannington, at Minalitlan, 18° North, Gulf Coast, Mexico. The history of the case is as follows :—

> ' Larvae of fly said to be a green fly by Mexicans, taken from Spaniard infected while drunk in field. Treated at native hospital for two weeks for catarrh. Came under observation delirious and with excessive oedema of glottis. Thirty-five large maggots removed by chloroform inhalations, and after three days a second (?) generation of smaller worms. Removed to railway

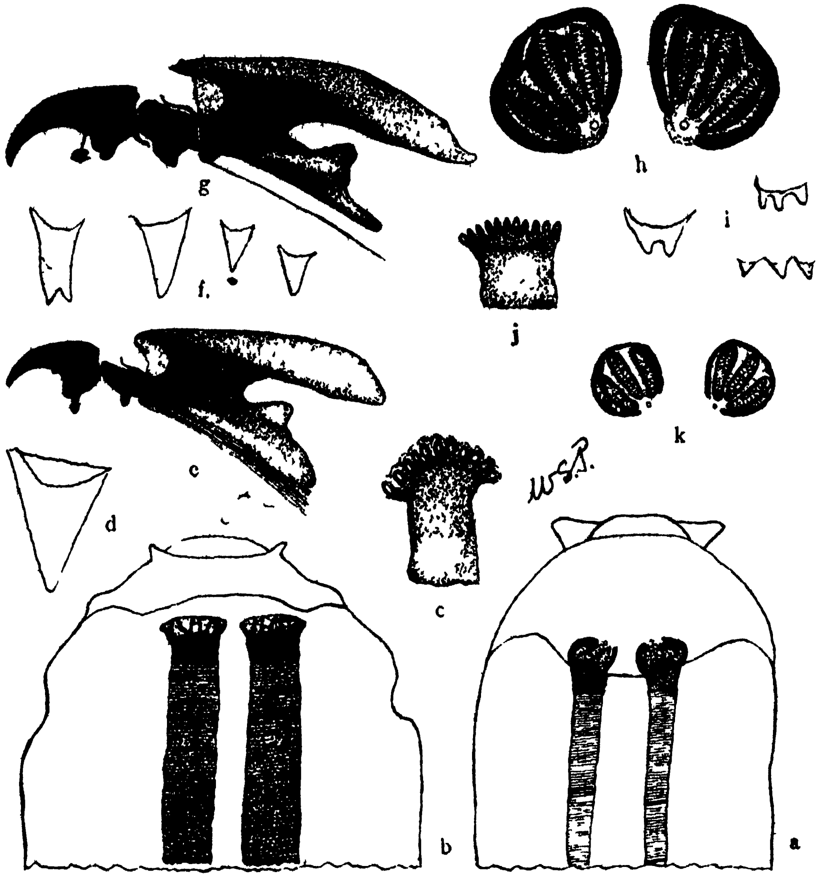

FIG. 7. *a.*—End of third larva of *macellaria*, to show non-chitinised tracheae; *b.*—Same of Mexican larva, to show chitinised tracheae; *c.*—anterior spiracles of Mexican larva; *d.*—Body spine of Mexican larva; *e.*—Cephalopharyngeal skeleton of third larva of *macellaria*; note ribbed pharynx; *f.*—body spines of Mexican larva; *g.*—Cephalopharyngeal skeleton of Mexican larva; note smooth pharynx; *h.*—Posterior spiracles of third stage Mexican larva; *i.*—Body spines of larva of *macellaria*; *j.*—Anterior spiracles of third larva of *macellaria*; *k.*—Posterior spiracles of same (all to same scale).

hospital where, four days later, after sharp haemorrhage, coughed up a large mass of larvae and made good recovery. Soft palate destroyed and bridge of nose. Eustachian tube affected; quite deaf on one side.'

This is the history of a typical case of nasopharyngeal myiasis, and it occurred to us that this larva may possibly be that of *americana*. The salient diagnostic characters of the cephalopharyngeal skeleton, anterior and posterior spiracles are illustrated in fig. 7, *g*, *h*, *f*, *c*, *b*, as well as those of the typical larva of *macellaria* (fig. 7, *e*, *i*, *k*, *a*), drawn to same scale for comparison. The differences in the structure of the cephalopharyngeal skeleton, posterior spiracles and dermal spines are so striking that there is no necessity to describe them. We would, however, draw particular attention to the fact that the pharynx of this Mexican larva is *smooth*, proving that it is a true parasite, whereas the pharynx of the larva of *macellaria* is *ribbed*, proving that it is not a parasite, but saprophytic in habit (obligatory necrobiot). It is interesting to note also that the main tracheal tubes (fig. 7, *b*) of the Mexican larva are wide and heavily chitinised for more than half their length, and can be seen through the skin, the atrium also being large, whereas those of the larva of *macellaria* (fig. 7, *a*) are much narrower and cannot be seen through the skin, and the atrium is much smaller. These striking characters again point to the Mexican larva's being a true parasite, whereas the larva of *macellaria* is not.

NOTES. The discovery of this new and quite unsuspected species of myiasis-producing Calliphorine raises a number of important points.

1. It is quite evident that *americana* has been entirely overlooked for the best part of 50 years or more, and as a result *macellaria* has been universally regarded as the screw-worm fly of the New World.

2. The fact that no adults of *macellaria* were found in collection B is a very striking one, and clearly points to its being a very doubtful myiasis-producing fly. The structure of the larva, especially that of the pharynx, confirms this view. It seems clear, therefore, that *macellaria* cannot in the future be known as the screw-worm fly of the New World. This popular name must now be transferred to *americana*. We suggest that *macellaria* is a harmless fly.

3. As far as we know at present, there is no evidence that *americana* breeds in decaying animal matter (meat, carcasses, etc.), and, although thousands of flies have been bred in this way throughout the States, no one has yet recorded *americana*. This at once suggests to us that *americana* is a true specific myiasis-producing Calliphorine and belongs to group 1, subgroup 2 (see classification of myiasis-producing Diptera in 'Insects, Ticks, Mites,' etc., Part I, Patton & Evans), and is, therefore, allied to the Old World *C. bezziana* Vill., which breeds only in living tissues. The mystery surrounding *americana* is exactly analogous to that which surrounded *bezziana* for many years, until one of us (W. S. P.) cleared up its true identity

and worked out its life history in India. Until then, *C. megacephala* (*flaviceps*, *dux*) was regarded as the important myiasis-producing Calliphorine of India, and was believed to be the adult of the larva very commonly found in human and animal tissues in that country. From hundreds of adults bred out by one of us (W. S. P.) in India from larvae collected by medical and veterinary officers from cases of human and animal myiasis, in only two cases were the adults of *megacephala* recovered, proving conclusively that this Calliphorine is of no importance as a myiasis-producing fly, and is, therefore, even a doubtful semi-specific species. In the same way, in the New World the true identity of the screw-worm fly has for long remained a mystery, owing (1) to the fact that the adults closely resemble those of *macellaria*, and (2) that, as it is almost certainly a true specific myiasis-producing fly and therefore never lays its eggs in decaying animal matter (carcasses, meat, etc.) but only in living tissues, the adults can only be obtained by breeding them out from third stage larvae. We fear that the important facts regarding the life history of *bezziana* have not been clearly appreciated.

4. We have no proof at present that any specimens of *americana* have been caught in traps or in places where blow-flies usually collect. We have little doubt that odd specimens of *americana* bred out from larvae from cases of myiasis exist in private and museum collections under the name *macellaria*. No one else to our knowledge has, in America, bred out *americana* in large numbers as recorded in the experiments noted above.

5. We have no evidence that *americana* has been described by any of the older authors, such as Macquart, Robineau-Desvoidy, Bigot, Wiedemann or Walker. We know that *macellaria* has been described many times by these older authors, and Walker described it at least seven times. In this connection, we would again emphasize the point that if our prediction that *americana* is a true specific myiasis-producing fly is correct—and we have overwhelming evidence to support this view—it is clear that the adults can only be obtained by either breeding them out or catching them while laying their eggs in wounds, etc. Mr. Cuthbertson (1933) in Rhodesia has recently recorded having caught about 50 females of *bezziana* when they were laying eggs on wounds in cattle, and notes that the adults are practically never seen otherwise. We doubt, therefore, whether any of the older

authors named above would have had an opportunity of seeing such bred adults, as very few observers who may have collected its larvae would have bred out the adults. We sincerely hope, therefore, that no dipterist will change the name *americana* for a doubtfully older name on the law of priority without first examining the male and female terminalia and comparing them with those of *americana*.

We should like to take this opportunity of drawing attention to the vital importance of accurate comparative studies of microscopical preparations of the male terminalia as the only certain means of settling, not only the true identities, but the relationships of the Diptera. The warning given by one of us (W. S. P.) in a previous paper (1933) should be carefully noted. We know of no better case with which to emphasize this point than the present one, where the neglect to examine the terminalia has led to the confusion of two species, one of which has been missed. One of us (W. S. P.) has already laid the foundation of this work, and these studies, which are now in active progress, will eventually lead, not only to more accurate information as to the identities and relationships of these flies, but to a natural classification. We would urge all dipterists, who use the characters of the male terminalia, to follow the terminology (of the parts), which has been introduced by one of us, and which is as near the truth regarding their homologies as is possible with our present knowledge.

In conclusion, we would like to point out that there are still those who regard these microscopic studies of the male terminalia as being only of academic interest, the argument being that the characters of the male terminalia are only those of one organ, the phallosome, and that they are of no use in the identification of the female. The characters of the female terminalia of the higher Diptera have yet to be explored. In the present case we have brought forward striking proof that the characters of the female terminalia are equally important, and can be used in the field for separating these two closely allied species, *macellaria* and *americana* (fig. 6).

SUMMARY

1. A new myiasis-producing Calliphorine, *Cochliomyia americana*, is described, whose identity has been obscured by its close resemblance to *C. macellaria* F. It is the true screw-worm fly

of the New World. The male and female terminalia are described and compared with those of *macellaria*.

2. It is suggested that it is a true specific myiasis-producing fly, like *C. bezziana*. A Mexican myiasis-producing Calliphorine larva is described, which may be the larva of *americana*.

3. Emphasis is again drawn to the importance of the study of the male and female terminalia in the higher Diptera.

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THE POLYMORPHIC TRYPANOSOMES OF DAMBA ISLAND, VICTORIA NYANZA

III.—THEIR REACTIONS IN LABORATORY ANIMALS

BY

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The first two papers in this series dealt with three strains of trypanosomes from wild *G. palpalis* on Damba Island, Lake Victoria, as regards their power of infecting man and their transmissibility by laboratory-bred tsetse (Duke, 1932*a* and *b*). This paper deals with :—

1. The length of life of various untreated laboratory animals infected with these strains.
2. The effect of human serum on strains Damba II and III in rats and mice.
3. The effect of atoxyl on strains Damba II and III in mice.
4. The results of red-cell adhesion tests with trypanosomes and plasmas from animals infected with strains I, II and III.
5. The presence of postero-nuclear forms.

1. THE LENGTH OF LIFE OF LABORATORY ANIMALS. *Strain Damba I*, first isolated in monkey 910, in February, 1932, was the *least* transmissible (Duke, 1932*b*) by *G. palpalis*, and was therefore not maintained so long as the other strains. Five monkeys in all were infected, the average life being 42 days, the maximum 109 days, the minimum 10. This last monkey was a feeble animal, and probably gives an exaggerated idea of the virulence of the strain ; if it is omitted from the series, the average life is 50 days. The strain was then kept up in guinea-pigs ; the average life of five was 26 days, the maximum 37, the minimum 14 days.

Strain Damba II, first isolated in monkey 911, also in February, 1932, was intermediate in transmissibility between strains I and III. Only two monkeys were infected with it, numbers 911 and 944. Monkey 911 lived 22 days and died of pneumonia ; monkey 944 lived 60 days. The strain was then kept up in guinea-pigs, a series of 15, the average life being 43 days, the maximum 114, the minimum 13 days. There was no obvious increase in virulence from the first to the last in the series.

Strain Damba III was isolated at the same time as the other two strains. The first monkey infected, number 912, lived 84 days. Eight other monkeys were infected from it by fly, the average life being 98 days, the maximum 163, the minimum 55 days. The strain is being kept up in guinea-pigs, the series to date, November 3rd, 1933, reaching 28. The average life was 34 days, the maximum was 78 days, the minimum 19; here again the virulence varied but did not constantly increase or diminish. Three untreated rats lived 97, 36 and 103 days. Two mice, also untreated, lived 45 and 172 days, and two sheep, infected by fly, lived 334 and 254 days. Two rats and one mouse were untreated but examined daily for the presence of postero-nuclear forms. They lived 18, 46 and 28 days.

The length of life of these animals may be compared with those used in experiments with strains of trypanosomes from Damba Island on two previous occasions (Duke, 1912 and 1921). In 1912, monkeys were infected with trypanosomes recovered from situtunga antelope on Damba Island. The actual length of life is not recorded, but one monkey was alive, though emaciated, after 124 days, others were alive and with no obvious symptoms after 85, 75, 70, 54 and 42 days.

In 1920, three strains were examined, one from wild *G. palpalis* on the mainland shores of Lake Victoria, a second from the Damba antelope, and a third from wild *G. palpalis* on Damba Island.

For the mainland strain, the average life of 12 monkeys was 216 days, and of 2 dogs 35 days. Two infected guinea-pigs were alive after 117 days, and [illegible] after 122 days.

For the Damba antelope strain, in 10 monkeys the average life was 53 days, in 2 guinea-pigs 41 days, and in 3 sheep 48 days.

For the Damba wild fly strain, the average life in 4 monkeys was 84 days; one guinea-pig lived 56 days, and another 158 days.

(The monkeys used in all these experiments were of the same species of *Cercopithecus* (*C. aethiops* Linn.). The rats and mice were from laboratory strains originally from England.)

It is difficult to make an exact comparison from these figures, but the strains to-day appear to be more virulent than those tested in 1912 or in 1920.

2. THE EFFECT OF HUMAN SERUM. Before the details of the experiments are given, some facts about the behaviour of the Damba strains in *untreated* animals must be considered.

After an animal has been inoculated, fresh or stained films made daily show no trypanosomes for the first 4-7 days. They are then to be seen in the stained film and later in the fresh film. They continue to be present for a period of some 4 or 5 days ; then they disappear, usually in both fresh and stained films, sometimes only in the fresh film. In addition to this first disappearance, which is variable in duration, there may be other negative periods quite irregularly distributed throughout the course of the infection.

This variation in numbers in the peripheral blood is in striking contrast to the behaviour of *T. rhodesiense*, which, at any rate in rats and mice, once it has appeared in the blood is present constantly till death. In guinea-pigs, however, even in *T. rhodesiense* and *T. brucei* infections, there may be periods in which no trypanosomes are seen. This is particularly important in connection with experiments in drug resistance, and will be discussed later. Thus, while it is comparatively easy to show that a given dose of serum has *no effect* on an infected animal, it is sometimes difficult to decide whether the disappearance of trypanosomes from the peripheral blood is accidental or directly due to the injection of serum.

In the following experiments, serum from three natives, 'A,' 'L' and 'K,' was used, sometimes fresh, sometimes dried *in vacuo* over sulphuric acid and redissolved before use in the requisite quantity of distilled water. The serum was always given intraperitoneally.

In all the Tables in this paper, each '+' or '—' sign, or the words 'very few' or 'few,' represents the result of blood examination on one day, the first sign referring to the day immediately following that on which the serum or drug was given. The sign 'O' means that the animal was not examined on that day ; 'very few' means that trypanosomes were found in a fresh cover-slip preparation only after 5 minutes' search. 'Few' means that from 1-10 fields of a one-sixth objective with 7x ocular were examined before finding a trypanosome, while '+' means any greater number than this. ***Every animal was + on the day on which the serum or drug was given.***

TABLE I

The effect of human serum on mice infected with strain Damba III

Animal	Dose of serum	Day after infecting inoculation on which serum was given	Result of fresh film examination on successive days following serum injection
Mouse 11	1·0 c.c. serum 'A' (fresh)	11th	— + + — +
„	1·0 „ „	24th	— — — — — — — — — — — — — — — — — — —
Mouse 5	0·5 c.c. „ „	5th	— — — — — — — — — — — — — — — — —
„	0·5 c.c. „ „	18th	died on 19th day—in fresh film.
Mouse 6	0·5 c.c. „ „	14th	+ — — — — —
Mouse 4	0·5 c.c. sheep serum	6th	+ + + + — + + + + + + + + +
„	0·25 c.c. serum 'A'	21st	— — — — — — — — — — — — — — — —
Mouse 9	0·5 c.c. „ „	14th	+ + + + + + + + + + + + + + +
Mouse 7	0·5 c.c. „ „	14th	— + + + + + + + + + + + + + + + + + +
Mouse 8	0·5 c.c. „ „	14th	— — — — + + + + + + + + — — — — +
Mouse 10	0·5 c.c. „ „	14th	+ + — — — + + + + + + + — — + + + + — + + + + + + +
Mouse 12	0·5 c.c. „ „	9th	— — — — — — — — — — + + — — + + — + + + + + + + + + + + + + + + + + + died

From this Table it appears that strain Damba III in mice, the only strain tested in these animals, is definitely susceptible to 1·0 c.c. and to 0·5 c.c. human serum. 0·25 c.c. proved effective in one animal (mouse 4), but it may have been reinforced in some way by the dose of sheep serum injected previously. A dose as small as 0·05 removed the trypanosomes for 10 days in one mouse (mouse 12), but had no effect on another (mouse 9).

Strains Damba II and III were tested in two series of rats; the results are given in the Tables II and III.

TABLE II

The effect of human serum on strain Damba II in rats

Animal	Dose of serum	Day after infecting inoculation on which serum was given	Result on days following injection
Rat 6	0·25 c.c. serum 'K' (fresh)	12th	few, — — — — +
Rat 5	0·5 c.c. „ „	9th	+, few, — — — +
Rat 1	0·5 c.c. „ „	9th	+ — — — — — +
Rat 2	0·5 c.c. serum 'L' (fresh)	13th	+ + +
„	1·0 c.c. „ „	17th	+ + +
„	2·5 c.c. „ „	21st	— — — — — — — — — — +
Rat 8	0·5 c.c. „ „	9th	few, — — — — — — — +
Rat 9	1·0 c.c. „ „	9th	— — — — — — — — +
Rat 12	1·0 c.c. „ „	9th	+ + + + + + + + +
Rat 3	0·5 c.c. serum 'A' (dried)	10th	+ + + + +
„	1·0 c.c. „ „	16th	+ + + +
Rat 4	1·0 c.c. „ „	10th	+ + + +
Rat 7	0·5 c.c. serum 'A' (fresh)	11th	+ — — — — — +
Rat 10	1·0 c.c. „ „	11th	+ — — — +
Rat 11	1·0 c.c. „ „	11th	— — — — — — — — — — +

TABLE III

The effect of human serum on strain Damba III in rats

Animal	Dose of serum	Day after infecting inoculation on which serum was given	Result on days following injection
Rat 2	0·25 c.c. serum 'A' (dried)	15th	few, — — — +
Rat 1	0·5 c.c. „ „	14th	— — — — — +
Rat 8	0·5 c.c. serum 'A' (fresh)	12th	+, few, few, few, +
Rat 9	1·0 c.c. „ „	12th	— — — — — — —, few
Rat 10	1·0 c.c. „ „	12th	— — — — — +
Rat 5	0·5 c.c. serum 'L' (fresh)	9th	— — — — — — —, few
Rat 6	1·0 c.c. „ „	9th	+ + +, died
Rat 7	1·0 c.c. „ „	9th	— — — — — — — —, few
Rat 4	0·25 c.c. serum 'K' (fresh)	10th	very few, —, few
Rat 3	0·5 c.c. „ „	9th	— — — +

Strains II and III are apparently both susceptible to human serum in rats, number III being on the whole rather more easily affected, particularly when tested with serum 'A.' Rats 3 and 4 (Damba II) were apparently unaffected by this serum, which in this experiment was used in the form of dried powder redissolved in distilled water. That the drying of the serum was not responsible is shown by comparing the result with rat 1 (Damba III), which was given dried serum, with rat 8, which had fresh serum; the dried serum had more effect. Serum 'L' brings out the difference between the two strains, 0·5 c.c. being effective in rat 5 (Damba III) but not in rat 2 (Damba II). Individual rats seem to vary in their power of reacting to human serum: rats 9 and 12 (Damba III) were both treated with serum 'L': one was affected, but the other apparently was not.

In contrast to the above results, it is interesting to note that rats inoculated by Duke with the situtunga strain from Damba in 1912 were unaffected by 1·0 c.c. of human serum, trypanosomes being present in fresh films of their blood for at least 6 days after the injection (Duke, 1912).

Duke also found that three strains, isolated by him in 1920 (Duke, 1921) and already referred to in Part I of this paper, were unaffected by human serum when tested in rabbits and guinea-pigs. The serum was given half an hour before the inoculation of trypanosomes or else mixed with them. The animals were not protected from infection.

3. THE EFFECT OF ATOXYL ON STRAINS DAMBA II AND III IN MICE. The effect of atoxyl on the two Damba strains II and III was tested in mice in the following way:—

The mice were inoculated intraperitoneally, usually from guinea-pigs, sometimes from other mice or from monkeys. Fresh blood films were examined daily.

As described in the last section, there is usually a negative period in the early days of the infection. As far as possible, the dose of atoxyl was not given till this negative period had been demonstrated. Some of the mice, however, were injected with atoxyl on the fifth day without waiting for the relapse to occur. There was not sufficient evidence to determine whether there was any difference in the sensitiveness of the strain to atoxyl before or after the relapse.

The atoxyl was given intraperitoneally in solution in water, the dose being adjusted to be within workable limits, that is, between 0·2 c.c. and 1·0 c.c. per mouse. The results are shown in the following Tables, which show that the Damba II strain is almost unaffected by 0·025 gms. per kilo., but is removed from the peripheral blood by 0·05 gms. With strain Damba III the results are somewhat contradictory: it appears to be slightly affected by 0·025 gms. per kilo. in 5 out of 7 mice, but 2 out of 5 mice that received 0·05 gms. per kilo. were unaffected. A dose of 0·1 gm. per kilo., however, removed trypanosomes from the peripheral blood for at least a week in all three mice tested.

TABLE IV

The effect of atoxyl on strain Damba II in mice

Animal	Dose of atoxyl	Day after infecting inoculation on which atoxyl was injected	Result of fresh film examination on successive days following injection
Mouse 3	0·025 gms. per kilo.	9th	+ + + + +
Mouse 4	0·025 gms. "	10th	+ + + +, few, +
Mouse 7	0·025 gms. "	9th	few, few, + +
Mouse 8	0·025 gms. "	11th	+ + +, died
Mouse 5	0·05 gms. "	10th	few, few, —, few, + +
Mouse 1	0·05 gms. "	9th	few, — — + +
Mouse 2	0·05 gms. "	9th	+ + + — — — +

Two monkeys infected with strain Damba III received doses of atoxyl ; they are mentioned here because it is interesting to compare their response to atoxyl with that recorded by Duke (1927). His five control monkeys, infected with a strain recovered from a situtunga from Damba Island in February, 1924, were given doses of atoxyl of 0·01, 0·028, 0·028, 0·028 and 0·02 gms. per kilo., and trypanosomes disappeared from the blood for 9, 19, 11, 10 and 9 days respectively.

The doses of atoxyl given to the two monkeys and their response to the drug were as follows :—

Monkey 1093 was given 0·03 gms. per kilo. on the eighteenth day after inoculation. It was positive on the nineteenth day, negative for 3 days, then relapsed.

Monkey 1106 was given 0·05 gms. per kilo. on the thirteenth day after inoculation, was negative for 10 days, and then relapsed.

These results indicate an increased resistance to atoxyl in the 1932 strain compared with that of the Damba strain isolated in 1924.

TABLE V

The effect of atoxyl on strain Damba III in mice

Animal	Dose of atoxyl	Day after infecting inoculation on which atoxyl was injected	Result of fresh film examination on successive days following infection
Mouse 6	0·025 gms. per kilo	18th	+, few, — —, few, + (— spontaneously on the 14th, 15th and 16th day)
Mouse 7	0·025 gms. „	9th	+ + + + + + + +
Mouse 12	0·025 gms. „	7th	+ + +
Mouse 14	0·025 gms. „	5th	+ + — O O +
Mouse 15	0·025 gms. „	5th	+, few, — O O +
Mouse 17	0·025 gms. „	5th	very few, —, few, few, +
Mouse 18	0·025 gms. „	5th	few, — —, few, +
Mouse 1	0·05 gms. „	14th	+ + + + + + + + +
Mouse 4	0·05 gms. „	9th	very few, — — — — — — +
Mouse 9	0·05 gms. „	16th	few, few, — — — —
Mouse 16	0·05 gms. „	5th	+ — — O O —
Mouse 19	0·05 gms. „	10th	+ + + + + + +
Mouse 2	0·1 gms. „	14th	— — — — — — — — +
Mouse 3	0·1 gms. „	14th	— — — — — — — — — +
Mouse 5	0·1 gms. „	9th	— — — — — — — — — — — died

4. SOME RED-CELL ADHESION TESTS WITH TRYPANOSOMES AND SERA FROM ANIMALS INFECTED WITH STRAINS DAMBA I, II AND III.

As explained in a previous paper (Duke and Wallace, 1930), experiments in red-cell adhesion, especially with *gambiense* strains, cannot always be made in accordance with any predetermined programme ; they have to be done when there are sufficient trypanosomes present in the peripheral blood of the infected animal. There are, therefore, unavoidable gaps in the following series. The technique used was that described by Wallace and Wormall (1931).

The following is a summary of the animals used :—

Strain Damba I : 3 guinea-pigs as source of trypanosomes ; 1 monkey supplying ' adhesin plasma.'

Strain Damba II : Trypanosomes from 1 monkey and 1 guinea-pig ; no animal available for adhesin plasma.

Strain Damba III : 3 monkeys, 1 guinea-pig and 1 mouse as sources of trypanosomes ; 5 monkeys supplying adhesin plasma.

The following Tables give the results of the tests. A ' positive ' test is one in which at least 50 per cent. of the trypanosomes show adherent red-cells.

TABLE VI

Adhesion tests with Damba trypanosomes

Trypanosomes	Plasmas				
	Homologous same strain	Other Damba strains	*gambiense*	*rhodesiense*	*brucei*
Damba I	2 positive 4 tested	0 positive 7 tested	0 positive 8 tested	0 positive 5 tested	0 positive 2 tested
Damba II	none tested	1 positive 7 tested	1 positive 6 tested	none tested	0 positive 5 tested
Damba III	1 positive 4 tested	1 positive 1 tested	2 positive 22 tested	0 positive 3 tested	1 positive 9 tested

TABLE VII

Adhesion tests with Damba plasmas

Plasmas	Trypanosomes				
Damba I	2 positive 4 tested	0 positive 3 tested	0 positive 1 tested	0 positive 8 tested	1 positive 7 tested
Damba III	1 positive 4 tested	1 positive 13 tested	0 positive 3 tested	3 positive 26 tested	8 positive 16 tested

No plasmas of strain Damba II were available

These Tables show that all three strains of trypanosomes reacted with their homologous plasmas ; Damba II and III also reacted with other Damba strains. Damba II trypanosomes reacted with *gambiense* plasma, but not with *rhodesiense* and *brucei* plasmas. Damba III strain reacted with *gambiense* and *brucei*, but not with *rhodesiense* plasmas.

Damba I plasma reacted with Damba I trypanosomes and in addition only with *T. brucei*.

Damba II plasma was not tested. Damba III plasma did not react with *T. gambiense*, but reacted with *T. rhodesiense* and with *T. brucei*. With *T. brucei*, 8 positive tests out of 18 tested were obtained using two strains of this trypanosome.

Fifty per cent. was regarded as the minimum degree of adhesion to justify a ' positive ' diagnosis in the clinical test (Duke and Wallace, 1930). This figure is more or less arbitrary, and was fixed high to exclude non-specific adhesion by a wide margin, although in plasmas from 100 normal natives these non-specific reactions never exceeded 6 per cent. of trypanosomes adherent in any one test.

Further support for the figure of 50 per cent. was given by the fact that, in a series of tests on sleeping sickness cases in the field, the positive reactions were all at least as high as this ; there were no uncertain responses.

Since this figure of 50 per cent. adhesion is arbitrary, we may lower the standard in the present series of experiments, in which we aim at detecting relationship between different strains and not the diagnosis of human trypanosomiasis.

Taking, therefore, 20 per cent. as the standard, instead of 50 per cent., we get a higher proportion of positive results with homologous strains and also an increase in the number of cross reactions. Thus Damba I plasma gives a reaction with *T. gambiense* (one positive, one test) and also with *T. rhodesiense* (one positive out of 8 tests), while Damba III plasma also gives one positive out of 3 tests with *T. gambiense*.

To sum up, red-cell adhesion experiments have demonstrated that there is some affinity between the Damba trypanosome and the three better-known polymorphic trypanosomes, *T. gambiense*, *T. brucei* and *T. rhodesiense*. There are insufficient tests for a strict comparison, but it must be remembered that a negative test does not necessarily mean that there is no relationship between the strains tested : it may be due to some interfering factor not yet recognised, possibly some anti-complementary substance, for it is practically certain that complement is necessary for appearance of the adhesion phenomenon (Wallace and Wormall, 1931).

Taking the results as they stand, the cross reaction for which there is the highest proportion of ' positives ' is that between strain Damba III and *T. brucei*; but we are not justified in taking this as proof of a closer relation between the Damba trypanosome and *T. brucei* than between it and the other two polymorphic trypanosomes, for the total number of tests is small, and previous work on adhesion (Duke and Wallace, 1930) suggests that a reaction with *T. brucei* or *T. rhodesiense* does not occur so readily, is more transient, and is not so specific as a reaction with *T. gambiense*.

5. THE PRESENCE OF POSTERO-NUCLEAR FORMS. An examination was made of stained films from the blood of two rats and one mouse, infected with strain Damba III, for the presence of postero-nuclear forms.

Mouse 21 was inoculated 22.6.33 and examined daily for nine days. Two hundred trypanosomes were counted per day, and no postero-nuclear forms were seen.

Rat 11 was inoculated 22.6.33 ;

On	29.6.33	200	trypanosomes	counted.	No	postero-nuclear	forms	seen.
„	29.6.33	200	„	„	3	„	„	„
„	30.6.33	200	„	„	2	„	„	„
„	1 6.33	200	„	„	5	„	„	„
„	3.6.33	200	„	„	30	„	„	„

Rat 12 was inoculated 22.6.33 ;

On	27.6.33	200	trypanosomes	counted.	No	postero-nuclear	forms	seen.
„	28.6.33	200	„	„	No	„	„	„
„	29.6.33	200	„	„	No	„	„	„
„	30.6.33	200	„	„	1	„	„	„
„	1.7.33	200	„	„	1	„	„	„
„	4.7.33	200	„	„	1	„	„	„
„	5.7.33	200	„	„	1	„	„	„
„	6.7.33	200	„	„	3	„	„	„

When first examined in 1912 (Duke, 1912), no search was made for postero-nuclear forms. In 1920, these forms were demonstrated in the mainland fly strain, Damba wild fly strain, and also in the strain from Damba antelope (Duke, 1921).

DISCUSSION

These Damba trypanosomes are interesting because they are the present-day representatives of the polymorphic group in a region that was the scene of an epidemic some 30 years ago and has since been uninhabited by man.

As pointed out by Duke (1912), these strains must either be the descendants of the *T. gambiense* that caused the epidemic, or some form of *T. brucei* from the cattle present on Damba with the native population. Investigation of the strain isolated in 1920 led to the hypothesis that prolonged adaptation of *T. gambiense* to antelope tends to give it the characteristics of *T. brucei* (Duke, 1921).

If the Damba trypanosomes *are* the descendants of the original *T. gambiense*, their attributes to-day, taken with those of the strains isolated in 1911 and in 1920, certainly support this hypothesis of the modification of *T. gambiense* by its prolonged sojourn in the antelope, for they are intermediate between typical *T. gambiense* and typical *T. brucei*, as shown by virulence, sensitiveness to human serum, and adhesion reaction.

Kleine (1928) found that, towards the end of an epidemic of trypanosomiasis rhodesiensis, the trypanosomes isolated were indistinguishable by laboratory tests from *T. gambiense*; and it seems reasonable to suppose that the converse change from *T. gambiense* to *T. brucei* may occur.

Further evidence for the change towards *T. brucei* of the trypanosomes in the lake shore fly, still on the assumption that it began as *T. gambiense* and not as *T. brucei*, is given by the 'Fishermen Experiment' mentioned in the introduction to this series of papers (Duke, 1932*a*), in which it was found that large numbers of Lake Victoria fishermen examined over a number of years, though necessarily exposed to *G. palpalis*, were not infected; and, finally, it has been shown by direct experiment on volunteers that the present-day Damba strain cannot infect man (Duke, 1932*a*).

If it is suggested that, on the contrary, the Damba trypanosome is not and never was *T. gambiense* but is a modified *T. brucei*, the obvious question is, why is it not typical *T. brucei*? Why should it be modified in the direction of *T. gambiense*, or indeed why should it be modified at all? It is no longer possible to prove the origin of the Damba trypanosome, but the possibility that *T. brucei* may adapt itself to man is of great practical and academic importance.

The suggestion (Yorke *et al.*) that the power of a trypanosome to invade man can be correlated with the possession of a resistance to human serum has been somewhat discounted by recent experimental work by Corson (1933), Fairbairn (1933) and Adams (1933); it seems certain now that the solution of the problem will only be reached by direct experiment on man.

SUMMARY

1. A record is given of the length of life of laboratory animals infected with three strains of polymorphic trypanosomes isolated from wild *Glossina palpalis* on Damba Island, Lake Victoria, in 1932. These strains were found to be slightly more virulent than strains isolated and tested in 1920.

2. Two of the strains were tested in rats and mice with human serum and found to be susceptible.

3. Two of the strains were tested with atoxyl in mice. The dose required temporarily to clear the peripheral blood was from 0·05 to 0·1 gms. per kilo. Strain Damba III in monkeys was found to be more resistant to atoxyl than a strain of Damba trypanosome tested in 1924.

4. Red-cell adhesion tests made with the three strains show relationships between them and also cross reactions with *T. gambiense*, *T. rhodesiense* and *T. brucei*. There is not sufficient evidence to determine which of these trypanosomes bears the closest relationship to the Damba strains.

5. The characters of the present-day Damba trypanosomes afford some evidence in support of Duke's hypothesis that sojourn in antelope may modify *T. gambiense* in the direction of *T. brucei*.

In conclusion, my thanks are due to Dr. H. Lyndhurst Duke, O.B.E., for the use of the three Damba strains isolated by him, and for his advice.

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STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

VII. *T. RHODESIENSE VERSUS T. GAMBIENSE*: A COMPARISON OF THEIR POWER TO DEVELOP CYCLICALLY IN GLOSSINA

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Until a few years ago, the programme of work at the Entebbe Laboratory was concerned mainly with *T. gambiense* and the nearly allied Damba trypanosomes. Serious study of *T. rhodesiense* first became possible on the arrival in Uganda of the League of Nations Commission in 1926, and it soon became evident that this trypanosome was readily transmissible by *G. palpalis*, a tsetse with which it does not commonly associate in nature (Duke, 1928). With increasing experience of its peculiarities, the impression gradually formed that *T. rhodesiense* was on the whole more readily infective to *G. palpalis* than the majority of strains of *T. gambiense* and of the Damba trypanosome hitherto examined in Uganda. Sufficient data have now been accumulated at the Institute to warrant an examination of the accuracy of this impression, and it is the main object of this paper to do this.

After a careful review of the figures, the method of presentation adopted below has been selected as the most informative. The three Tables show data relating to the transmissibility, at the time of or shortly after their first isolation, of a number of strains of *T. gambiense*, *T. rhodesiense* and tsetse-carried polymorphic trypanosomes from sources other than man (in other words, *T. brucei*). The data are set forth according to the method adopted in the Final Report of the League of Nations Commission (Duke, 1928). Only the earlier passages of each strain are included, and the figures obtained with each animal of these passages are given separately.

Many of the totals—especially those of earlier experiments—are obviously too small to give any but a very rough idea of the transmissibility.

The clean flies used in these tests were applied in boxes of 50 or so, the flies of each box feeding twice, with 48 hours' starvation between the feeds. As far as possible, feedings were arranged so that at least a batch of flies fed on the animal each day for some 7-10 consecutive days. Information is gradually being collected about Robertson's views on the endogenous cycle in the vertebrate (Robertson, 1913), but all investigators are agreed that the infectivity to Glossina of any animal's trypanosomes often varies greatly from day to day.

There is now no longer any serious doubt that strains of polymorphic trypanosomes do differ in their ability to utilize tsetse, and the systematic study of this particular attribute will assuredly enlarge our understanding of these parasites. The figures in the Tables do not represent by any means all the data accumulated at Entebbe, but they are sufficient for the purpose of this study. The strains were not specially selected, and, as stated above, only the earlier passages after their original isolation are included. Many of these figures have already appeared in previous publications.

The transmissibility indices given in the Tables are calculated from the formula :

$$T = \frac{s}{p} \times \frac{n \times 100}{N}$$

where T= *transmissibility index* ; N = total number of flies dissected ; n = total number of flagellate-containing flies ; p = number of infected flies of 25 days old or over; and s = the number of these mature infected flies that show gland infections. The total percentage of infected flies is called the *infectivity index* ; the fraction $\frac{s}{p}$, the *metacyclic index*.

In the Tables, three points should be specially noted. First, in column 5, the total percentage of positive flies produced by each strain (*infectivity index*) ; secondly, in columns 6 and 7, the proportion of flies, 25 days old and more, which have gland infections (*metacyclic index*) ; and thirdly, in column 7, the *transmissibility index*. It will

TABLE I.—*T. gambiense*

Strain No.	Infecting animal	Total number of flies dissected	Number infected	Percentage infected (infectivity index)	Transmissibility index	Glands infected / Flies alive on 25th day	Passage from man	Single tests	
								Number of flies	Percentage infected
I.	m. 52	216	6	2·7	0·9	1/3	1st	47	6·3
	80	88	1	1·1	0	0/1	3rd	45	2·2
II.	m. 53	321	22	6·8	0·4	1/15	1st	29	20·6
	sh. 332	262	6	2·2	0	0/5	2nd	31	9·7
	g. 340	178	13	7·7	0·6	1/12	2nd	43	16·2
III.	m. 113	196	29	14·7	0	0/26	2nd	57	21·0
	sh. 336	351	27	7·6	0	0/26	2nd	42	21·4
	m. 90	127	1	0·7	0	0/0	2nd	47	2·1
IV.	Man	155	6	3·8	1·8	3/6	...	37	8·1
	g. 339	134	11	5·7	1·0	2/11	1st	47	19·1
	g. 342	316	11	3·4	0	0/0	2nd	48	8·3
V.	m. 64	154	8	5·0	5·0	7/7	1st	32	9·3
	95	89	15	16·8	6·7	5/15	2nd	44	22·7
	sh. 333	150	9	6·0	4·2	5/7	2nd	42	9·5
	sh. 161	252	27	10·1	5·5	12/22	3rd	57	26·3
	sh. 217	224	12	5·3	3·1	3/5	4th	41	14·6
VI.	m. 65	150	4	2·6	0	0,3	1st	36	5·5
	g. 341a	235	6	2·5	0	0/5	2nd	29	6·9
VII.	Man	180	13	7·2	3·9	6/11	...	45	13·3
	sh. 335	173	7	4·0	0·6	1/6	1st	57	7·0
	g. 337	233	14	6·0	0·4	1/13	1st	37	10·8
	g. 330	224	3	1·3	0	0/2	1st	61	3·2
VIII.	Man	183	3	1·6	0	0/3	1st	45	6·6
	m. 120	204	9	4·4	0	0/5	1st	57	10·5
IX.	Man	158	16	10·1	7·9	11/14	1st	48	12·5
	g. 171	192	4	2·0	1·0	2/4	1st	41	4·8
X.	m. 149	158	4	2·5	0·6	1/4	1st	49	4·0
	g. 338	126	8	6·3	1·8	2/7	2nd	...	...
XI.	m. 162	218	16	7·3	3·3	5/11	...	57	10·5
XII.	Man	157	3	1·9	1·9	1/1	...	58	5·1
XIII.	Man	139	3	2·1	2·1	3/3	...	43	6·9
XIV.	Man	135	7	5·1	3·4	4/6	...	43	6·9
XV.	Man	149	7	4·6	4·6	5/5	...	45	6·6
XVI.	Man	187	6	3·2	1·6	2/4	...	46	6·5
XVII.	Man	177	1	0·5	0	0/0	...	42	2·1
XVIII.	Man	182	0	0	0	0	...	...	...
XIX.	Man	175	...	...	...	...	...	...	...
XXVII.	Man	123	10	8·1	4·8	3/5	...	50	4·0
	m. 407	319	5	1·5	0·8	1/5	1st	46	4·3
	m. 442	696	14	2·0	1·0	6/12	2nd	40	10·0

TABLE I—*T. gambiense*—*Continued*

Strain No.	Infecting animal	Total number of flies dissected	Number infected	Percentage infected (infectivity index)	Transmissibility index	Glands infected / Flies alive on 25th day	Passage from man	Single tests	
								Number of flies	Percentage infected
XXVIIA.	m. 479	767	4	0·5	**0**	0/1	1st	40	2·5
XXVIII.	m. 487	533	14	2·6	**1·8**	5/7	1st	47	8·5
XXVIIIA.	m. 488	338	10	2·9	**0·9**	2/6	1st	36	13·8
XXIX.	Various (E)	2255	40	1·7	**0**	0/30	various	...	...
XXX.	Man	332	16	4·8	**3·2**	8/12	...	53	7·5
	m. 503	175	11	6·2	**3·1**	4/8	1st	27	14·8
XXXI.	Man	307	11	3·5	**3·0**	6/7	...	49	6·1
XXXII.	Man	313	15	4·7	**3·9**	10/12	...	55	6·9
	m. 514	347	13	3·7	**2·1**	4/7	1st	43	9·3
XXXIII.	Man	243	13	5·3	**4·7**	9/10	...	43	13·9
	m. 516	163	12	7·3	**3·6**	3/6	1st	57	12·2
	m. 544	317	11	3·4	**1·3**	2/5	2nd	45	11·1
XXXIV.	Man	210	6	2·8	**2·8**	6/6	...	50	6·0
	sh. 28	285	6	2·1	**0·7**	1/3	1st	40	5·0
	sh. 12	534	4	0·7	**0**	0/3	1st	48	4·1
XXXV.	Man	236	1	0·4	**0**	0/1	...	43	2·3
XXXVI.	m. 554	456	3	0·6	**0**	0/3	1st	...	...
XXXVII.	m. 641	1050	1	0·01	**0**	0/0	1st	...	...
XXXVIII.	m. 664–5	855	17	1·9	**1·2**	10/15	1st	...	...
XXXIX.	Man	262	7	2·6	**2·0**	4/5	...	40	5·0
LI.	1090	333	3	0·9	**0·9**	2/2	1st	39	2·5
	1092	442	9	2·0	**1·5**	6/8	1st	40	7·5
	Oribi III	332	4	1·2	**1·2**	1/1	...	41	4·8
LII.	1095	266	10	3·7	**3·7**	10/10	1st	29	17·2
	1098	324	16	4·9	**4·0**	9/11	1st	20	25
LVII.	Various	551	21	3·4	**2·9**	12/14	1st	48	12·5

be seen that a strain (such as no. III) may be readily infective to fly and yet produce no gland infections. Again, in some strains, such as *T. gambiense* XV and *T. rhodesiense* L in the Tables, all flies living 25 days or longer have gland infections. But in the great majority of strains some of these old infected flies have no flagellates in their glands.

In the fraction denoting the *metacyclic index* (column 7) the denominator is the number of infected flies 25 days old or older,

TABLE II—*T. rhodesiense*

Strain No.	Infecting animal	Total number of flies dissected	Number infected	Percentage infected (infectivity index)	Transmissibility index	Glands infected Flies alive on 25th day	Passage from man	Single tests	
								Number of flies	Percentage infected
Mw. I.	m. 468–9	421	31	7·3	5·7	22/28	2nd	60	15·0
Mw. II.	m. 473	120	9	7·6	6·7	8/9	2nd	61	11·4
XX.	g. 143	134	9	6·7	5·5	5/6	2nd	39	15·3
	sh. 144	156	14	8·9	6·3	10/14	2nd	47	12·7
	g. 176	163	23	13·4	3·0	5/13	2nd	40	17·5
XXI.	m. 281	142	11	7·7	3·8	1/2	2nd	32	18·7
XXII.	m. 282	124	7	5·6	2·8	1/2	2nd	50	8·0
XXIII.	m. 353–4	521	19	3·6	3·0	11/13	2nd	47	8·2 (treated)
	m. 278	241	10	4·1	2·7	2/3	3rd	55	3·6
XL.	Man	261	11	4·2	2·8	2/3	...	38	7·9
XLI.	m. 376	219	4	1·8	1·8	2/2	2nd	48	4·1
	g. 174	201	3	1·4	1·4	1/1	3rd	44	4·5
XLII.	m. 377	276	7	2·5	2·5	4/4	2nd	45	4·4
	g. 214	207	7	3·3	1·1	1/3	3rd	47	4·2
	m. 397	213	16	7·5	7·5	8/8	4th	39	15·3
	m. 408	121	11	9·0	6·4	5/7	5th	31	16·1
XLIII.	m. 770	209	18	8·6	2·3	5/7	2nd	37	13·5
XLIV.	m. 771	774	68	9·3	5·3	22/38	...	61	19·6
XLV.	m. 772	341	26	7·6	6·7	8/9	...	41	14·6
XLVI.	m. 773	648	59	9·1	7·8	16/30	...	59	15·2
XLIX.	m. 972	627	47	7·6	3·5	14/30	...	37	21·6
L.	m. 1010–1	337	10	2·9	2·9	3/3	...	35	14·2
T1.	m. 949	188	16	8·5	7·3	13/15	3rd	50	20·0
	Reedbuck	472	39	8·2	1·2	4/26	4th	44	20·4
	m. 1015	233	20	8·5	3·8	5/11	5th	22	59·0
	m. 1040	297	49	16·5	8·2	9/18	6th	49	24·4
T2.	m. 950	289	26	9·0	8·2	11/12	...	46	21·7
T3.	m. 923	440	30	6·8	6	15/17	...	50	22·0
TX.	m. 956	257	4	1·5	1·5	3/3	2nd	52	3·8
TY.	m. 958	294	7	2·3	1·1	2/4	...	47	4·2
TZ.	m. 954	293	12	4·0	1·7	3/7	...	42	9·5

and the numerator shows how many of these had gland infections. It will be noticed that the ***transmissibility index*** cannot be greater than the total percentage of infected flies (***infectivity index***); also that there are strains whose general infectivity is low, notwithstanding

TABLE III—*T. brucei*

Strain No.	Infecting animal	Total number of flies dissected	Number infected	Percentage infected (infectivity index)	Transmissibility index	Glands infected / Flies alive on 25th day	Single tests	
							Number of flies	Percentage infected
E.A.	Various	1068	46	4·3	**3·0**	...	...	...
*p*D.A. 1920	m. 173	272	7	2·5	**1·2**	2/3	34	5·8
Mw. III.	Various	535	25	4·6	**3·5**	14/18	64	10·9
*p*XXII.	Various	958	2	0·2	**0**	0/0	...	...
XXIV.	m. 294	164	7	4·2	**2·8**	2/3	30	10·0
XXVI.	m. 336–7	378	7	1·8	**0·7**	2/3	43	4·6
*p*XLVIII.	m. 387	492	17	3·4	**1·7**	3/6	35	5·7
	m. 395	276	16	5·8	**3·4**	6/10	28	14·2
	g. 341	191	12	6·2	**3·4**	5/9	40	12·5
	sh. 300	210	11	5·2	**3·2**	5/8	37	13·5
	m. 414	220	10	4·5	**3·1**	5/7	27	11·1
LIII.	m. 889	639	11	1·7	**1·2**	3/4	31	6·4
	m. 894	402	9	2·2	**1·1**	1/2	50	6·0
	m. 915	636	31	4·8	**3·9**	18/22	52	17·3
	m. 948	416	15	3·6	**3·1**	7/8	51	7·8
LV.	m. 981	137	11	8·0	**4·5**	4/7	31	12·9
*p*LVI. (*a*)	m. 910	409	8	1·9	**1·3**	5/7	56	5·3
*p*LVI. (*b*)	m. 911	304	9	2·9	**2·9**	1/1	49	6·1
*p*LVI. (*c*)	m. 912	417	32	7·6	**5·0**	12/18	62	17·9
LVII.	m. 973	260	6	2·3	**0·9**	2/5	52	5·7
	m. 983	167	11	6·5	**4·3**	4/6	41	12·1
	1018	191	11	5·7	**4·9**	6/7	50	12·0

In this Table, the letter *p* indicates that the strain was derived from wild *G. palpalis*, *not* game tsetse.

that every infected fly of 25 days old or more has its glands infected (e.g., *T. gambiense* XII and *T. rhodesiense* XLI).

The two right-hand columns in the Tables relate to single boxes of flies. From each test on an animal the box was selected which showed the highest total percentage of infected flies, irrespective of, but including, gland infections. These two columns thus show the highest infectivity manifested by the strain during the period of the endogenous cycle covered by the test.

It must be remembered that the object of the Tables is to show whether the three principal polymorphic trypanosomes differ in

TABLE IV

Summary of essential data in Tables I, II and III

	T. gambiense	*T. rhodesiense*	*T. brucei*
Total number of strains	37	21	13
No. transmissible	26 = 71 per cent.	21	12
No. non-transmissible	11 = 29 per cent.	0	1 = 7·6 per cent.
Average per cent. of infected flies per strain	In the 35 strains that infected tsetse = 4·4 per cent. In the 26 transmissible strains = 4·2 per cent.	6·9 per cent.	In the 12 that infected tsetse = 4·6 per cent. For strains originating from game tsetse region (7) = 4·9 per cent.
Average transmissibility index per strain	2·5	4·7	3·0 Of strains from game tsetse region (7) = 3·6
Single tests—			
Total number	64	31	20
Number giving more than 10 per cent. of infected flies	22 = 34·3 per cent.	20 = 66 per cent.	11 = 55 per cent.
Number giving more than 15 per cent. of infected flies	9 = 15 per cent.	15 = 48 per cent.	2 = 10 per cent.
Number giving more than 20 per cent. of infected flies	5 = 8·4 per cent.	7 = 22·5 per cent.	0

their ability to develop in Glossina, and for this purpose the highest figures supplied by a strain have a special importance—even though the totals involved be small.

In addition to their main theme, the Tables suggest that individuals of a mammalian species may differ in the effects they produce on the transmissibility of a trypanosome : a strain developing in one individual may find itself better able to utilize the tsetse than the same strain living in another. Such differences in vertebrate hosts are not apparently associated with any recognisable clinical manifestation or syndrome, and experience has shown that the inhibitory influence of an individual host is often restricted to that host, especially in chronic infections. Change from one species of host animal to another may also be attended by great changes in the transmissibility of a strain. Indications of this kind occur so frequently in the records of the laboratory that they cannot be due entirely to chance.

DISCUSSION

The Tables given in this paper show that, for the strains therein examined, *T. rhodesiense* is as a general rule more readily transmitted by *G. palpalis* than *T. gambiense.* Some ideas suggested by this conclusion will now briefly be discussed.

G. palpalis is not the normal intermediary of *T. rhodesiense*, the tsetse responsible for carrying this trypanosome in East and Central Africa being *G. morsitans* and *G. swynnertoni* ; and of these two flies there is reason to believe that *G. morsitans* is a better carrier of trypanosomes than *G. palpalis* (Duke, 1933). It is therefore interesting to find that *T. rhodesiense* is better adapted to the *G. palpalis* of Uganda than strains of *T. gambiense* which depend upon this fly for their spread in nature. Whether this superiority will survive repeated cyclical passage through *G. palpalis* is a matter that is now under investigation.

The adaptation of *T. rhodesiense* to Glossina may be due solely to long dependence on a species of tsetse which offers relatively little opposition to the evolving trypanosome in its efforts at establishment in the fly, or it may result from more intricate adjustments between the parasite and both its intermediate and its vertebrate hosts. But, whatever be the progress made by members of the polymorphic group of trypanosomes in their adaptation to Glossina, there is no question that they have attained a very high degree of adjustment to the wild ruminants upon which the game tsetses feed, and any attempt to estimate the efficiency of a trypanosome in nature must take into account its relation to both its hosts.

We can with comparative ease determine experimentally—by transmitting it cyclically for a number of generations—whether a species of tsetse with which a trypanosome is normally unfamiliar can produce any change in the characters of the protozoan. If, for example, when a poorly transmissible *T. gambiense* is transmitted by *G. morsitans* an increase in transmissibility results, then the difference between *T. gambiense* and *T. rhodesiense* revealed in the Tables of this paper is easily intelligible, and we can accept the simple explanation that *T. rhodesiense* is merely *T. gambiense* that has learnt to reply on a game tsetse for its spread in nature. But difference in transmissibility is by no means the only difference between *T. rhodesiense* and *T. gambiense* ; indeed, in all its characters, save pathogenicity to man, *T. rhodesiense* resembles *T. brucei* more closely than it does *T. gambiense.* If, then, the tsetse—*G. morsitans* or perhaps some other game tsetse—is alone responsible for the alteration from one type of trypanosome to the other, it has much to change.

If, on the other hand, *T. rhodesiense* is really *T. brucei* in man's blood, then its superior adaptation to tsetse and all the other characters by which it differs from *T. gambiense* are at once explained. An ample and readily accessible supply of wild ruminants, well suited to the requirements of the trypanosome and in intimate contact with the insect intermediary, have produced in the course of ages a well-balanced and flourishing parasite, '*T. brucei,*' adjusted to both intermediate and definitive hosts. The only distinction existing between the widespread and successful *T. brucei* and the trypanosome known as *T. rhodesiense* is that of pathogenicity to man —an arbitrary distinction involving the rash assumption that this quality is a fixed character of the trypanosome.

And now a word about this power of invading man, to which such exaggerated importance has been attached. Investigations still in progress at the Institute have shown in the last few weeks that *T. rhodesiense* may in certain circumstances *lose this power.*

Evidently, therefore, pathogenicity to man—a character to which specific value has been attached by some workers—is not a deep-seated and essential feature in the organisation of this trypanosome ; and consequently it is not entitled to the respect which it has hitherto received.

Accordingly, there is no obvious obstacle to the assumption that, in certain circumstances, a strain of trypanosomes originally non-pathogenic to man may learn to overcome this disability, should the exigencies of its own existence demand it.

If we consider the evolution of a trypanosome towards the ideal of a blood parasite—harmless to its vertebrate host and readily transmissible by its invertebrate intermediary—there seems to be no obvious biological incentive for the tsetse, *per se*, to vary in its transmitting power. The onus of improvement is clearly on the parasite, which confers no apparent benefits on either of its hosts. The fact—if it be confirmed—that the different species of Glossina differ in their ability to transmit trypanosomes may well influence the progress of the evolution of the parasites. For example, *ceteris paribus*, a trypanosome will have better opportunities of adapting itself to its vertebrate hosts where the tsetse is naturally a good transmitter. The existing adaptation of the three polymorphic trypanosomes to antelope must surely be ascribed to natural selection acting for ages of time ; and here selection will tend to modify both vertebrate and trypanosome, for adjustment is equally important to both.

If, then, *T. rhodesiense* is merely *T. gambiense* circulating in man (and in game) by the agency of game tsetses instead of by its normal carrier, *G. palpalis*, surely it is remarkable to find such great changes in the characters of the trypanosome appearing in so comparatively short a time. For, although the first appearance of trypanosomiasis rhodesiensis in man in Northern Tanganyika may, it is true, date back a year or two further than is generally believed, native testimony makes it almost inconceivable that the disease has been prevalent in man for any long period of time.

The first strain examined from the Mwanza-Ikoma area of Northern Tanganyika Territory proved readily transmissible by *G. palpalis* and behaved in other respects like typical *T. gambiense*, when examined at Entebbe in 1922 (Table II, nos. 1 and 2) ; and the same was true of another strain from the same area sent to the Commission by Dr. Corson in 1927 (Lab. no. XX). The epidemic was first definitely recognised in 1920. Early in 1918, Dr. Andrew Balfour saw a slide taken from a case of human trypanosomiasis in Mwanza hospital, and a case from this area suggestive of—but not proved to be—human trypanosomiasis died in Mwanza hospital early in 1919 (Duke, 1923).

It is true that Kleine in 1927 found among a number of strains of *T. rhodesiense* examined in the area some whose virulence in laboratory animals resembled that of *T. gambiense.* But changes in virulence are known to occur in comparatively short periods of time. The only available information about the transmissibility of Kleine's strains is that given by nos. XXI and XXII of Table II, the number of flies used being small, with both the strains and the period covered by their investigation very short.

Diminution of the virulence of a trypanosome towards the end of the epidemic is to be expected if the parasite is making any progress in its efforts to adopt man as a host (Duke, 1930).

But, if *T. rhodesiense* is *T. brucei* established in man, one would expect to recognise the signs of its age-long association with the game and their tsetses in the form of ready transmissibility by tsetse and a high degree of adaptation to its game hosts. Recent work on *T. rhodesiense* at Entebbe has demonstrated in a very striking manner its possession of this latter quality, and the figures in Table II testify to its infectivity to tsetse. *T. gambiense* has never really succeeded in adapting itself to man in the way that *T. brucei* has to game, a mark of this failure being the appalling death roll due to sleeping sickness in past years. Where its contact with man is most ancient (as in certain parts of West Africa), there the adjustment to man reaches its highest development. Very recently a strain has been received from the extreme south of the Belgian Congo which suggests that here also a high degree of adaptation to man has been attained. I owe the opportunity of examining this strain to the commend e initiative of Dr. Barlovatz, of Tshikapa, Kasai, and to the Congo and Tanganyika authorities for ensuring its safe passage across some 1,600 miles of country. Dr. Barlovatz writes (4.1.33) that he has recently re-examined 5 natives who in July, 1929, had all been found infected with *T. gambiense,* trypanosomes being found in their lymphatic glands. All were untreated, and when examined in October, 1932, were apparently in perfect health; their cerebro-spinal fluid was normal, and a careful clinical examination failed to reveal any signs of illness. In two of them centrifugation of the blood revealed scanty trypanosomes, in one on the fourth and the other on the fifth centrifugation. Unfortunately, only one of these strains reached Entebbe, in a guinea-pig; the other animals inoculated at Tshikapa failed to take, a failure in keeping with my own experience of attempting to isolate mild *T. gambiense* strains from man. The patient supplying this strain, a woman of 50, was examined on 15 successive days by triple centrifugation of the blood, and on one occasion only were sparse trypanosomes seen. Her general condition was good, and there were no enlarged glands or other symptoms of illness. The C.S.F. was normal. During her period of examination the patient had an epileptic seizure. This strain is not included in the Tables, as its diagnosis is not yet quite certain. In addition to its mildness in man, the strain is readily transmissible by the Lake Victoria *G. palpalis*, a total of 551 flies showing *infectivity*, *metacyclic* and *transmissibility indices* of 3·4 per cent., 12/14 and 2·9 respectively. The highest percentage for an individual experiment was 12·5 per cent. in 48 flies. On its first arrival, the strain was remarkable for its great virulence in rats and mice, but this is diminishing after 4 months at the laboratory. In 4 monkeys the average duration of the disease was 35 days. The strain in guinea-pigs showed posterior-nuclear forms up to 2 per cent. of all trypanosomes counted in rats, and up to 5·5 per cent. in mice. Lastly, when examined by the adhesion test the strain responded in high titre to *T. gambiense* elements—serum or plasma or trypanosomes, as the case might be—but there was also some response to *T. rhodesiense* plasma, though not in so pronounced a degree. Here, then, is a strain of *T. gambiense*, in some ways atypical, but possessing apparently a high degree of adjustment to man.

This strain illustrates yet another point of great interest, upon which attention has been focussed of late both in East and West Africa, viz., the possession of considerable natural resistance to arsenic—a quality possessed by both *T. brucei* and *T. rhodesiense*. Thus, in rats, doses up to 0·1 grm. per kilo. had no effect, and 0·2 and 0·3 grm. per kilo. led to a disappearance of trypanosomes from the peripheral blood for 4-5 days only. At the moment of writing, full information is lacking about the species of tsetse occurring in this remote district, but the case was regarded by the Congo authorities as one of trypanosomiasis gambiensis, and presumably the strain was carried by *G. palpalis.*

Dr. L. Van Hoof informs me by letter from Leopoldville that he also is finding strains of *T. gambiense*, isolated from untreated natives along the River Congo, that possess considerable natural drug resistance, in one case even against ‘ Bayer 205.’

On the West Coast, an examination of a number of strains isolated from man in sleeping sickness areas, and all hitherto regarded as *T. gambiense*, has shown that some of them resemble *T. rhodesiense* in certain important respects. Three species of tsetse come within the purview of the West Coast investigators, *G. morsitans*, *G. tachinoides* and *G. palpalis*, the last two being known to spread human trypanosomiasis. *G. morsitans* has not yet been found in West Africa to feed on reptiles ; *G. tachinoides* and *palpalis* both feed on reptiles and on man, and the former especially also feeds on game animals.

There are thus excellent opportunities here for interchange of polymorphic trypanosomes between game and man, and *vice versa*, by means of two (and possibly three) different species of tsetse. And, as noted above, the strains of human trypanosomes show considerable variety, some resembling *T. gambiense*, others *T. rhodesiense.* Taylor's recent work on the transmission of human trypanosomes by *G. tachinoides* suggests that this tsetse is an excellent cyclical transmitter, better considerably than the *G. palpalis* of Lake Victoria.

The differences between these strains in their response to arsenic and human serum and in their general virulence suggests that there are present in man in these West Coast regions a number of different types ranging from typical *T. gambiense* on the one hand to typical *T. rhodesiense* on the other. And in the game there occurs also *T. brucei.*

Two explanations of this state of affairs suggest themselves. Either all these human strains are varieties of one human trypanosome, zoologically distinct from *T. brucei*, their differences being due to the influence of the species of mammal on which they chiefly depend in nature and on the tsetse that is mainly responsible for carrying them. Or they may all be strains of *T. brucei* that have for longer or shorter periods adopted man as their principal host—the pioneers being typical *T. gambiense*, the later invaders showing at once their origin and their inexperience by their *T. rhodesiense* characters.

The practical indication emerging from this discourse is the need for repeated experimental cyclical passage of *T. gambiense*—preferably of a feebly transmissible strain—through a game tsetse, to see whether any enhancement occurs in the transmissibility of the trypanosome and its virulence for small animals. The reverse experiment of passing *T. rhodesiense* and *T. brucei* through *G. palpalis* has been in progress for some 18 months at Entebbe, and the results will be published shortly. Suffice it to say here that, as regards certain attributes of trypanosomes (arsenic resistance, the appearance of posterior-nuclear forms, and transmissibility by tsetse), no definite change was apparent after some 10 cyclical passages carried out at the Laboratory. It will perhaps be objected that 18 months or two years—or whatever be the duration of the experiment—is not long enough ; nevertheless, it is a test of stability that a trypanosome can never conceivably undergo in nature, where a constant intermingling of strains is going on in every fly belt and probably in every adult antelope. If the tsetse proves able to influence the characters of the trypanosomes which it carries, then we have a simple explanation for the appearance of *T. rhodesiense* in a game tsetse belt, namely, the introduction of *T. gambiense* in man and its gradual conversion into the more virulent form. But if prolonged cyclical passage through *G. morsitans* produces no noticeable alteration in *T. gambiense*, then we must abandon this view for the much less easily demonstrable alternative that *T. gambiense, T. brucei* and *T. rhodesiense* are all varieties of one widespread species, their differences being due to the species of vertebrate on which in the course of time they have become mainly dependent.

Kleine's view is that *T. rhodesiense* and *T. gambiense* are one species zoologically distinct from *T. brucei*, *T. rhodesiense* being the form taken by *T. gambiense* when introduced into a new and non-immune population (Kleine, 1928). It is difficult, if not impossible, to test out this view on man himself, and a parallel test in any other animal is not enough. But it is perhaps worthy of note that in the occasional instances where Europeans contract trypanosomiasis in a *palpalis* area the trypanosome maintains the characters of *T. gambiense.*

The fact, then, that *T. rhodesiense* appears better adapted to *G. palpalis* than is *T. gambiense* suggests to the writer that *T. rhodesiense* is indeed *T. brucei* that has but recently taken to man, forced thereto by the opening up of Africa and the disturbance of primitive conditions. In this connection it is interesting to note Nash's observations on the distaste shown under normal conditions by the females of *G. morsitans* for man's blood, a dislike which is only overcome when game animals fail and the fly are seriously pressed for food (Nash, 1933). It is nowadays becoming increasingly evident that research in the future must attend particularly to man's reaction to the trypanosomes of the polymorphic group. We have to find out why he resists the majority of game trypanosomes—a difficult problem indeed. We have also to test far more freely than has been possible until recent years wild strains of the polymorphic group on man. And, lastly, we have to determine how permanent and deep-seated is the quality of a polymorphic trypanosome that enables it to use man as a host.

On solution of all these conundrums depends to a very great extent the future of large tracts of Africa.

SUMMARY

1. An examination of the Entebbe records of experiments on the transmission of *T. gambiense*, *T. rhodesiense* and *T. brucei* by *G. palpalis* (and to a less extent by *G. morsitans*) shows that *T. rhodesiense* is generally more readily cyclically transmissible by tsetse than *T. gambiense.*

2. The examination of strains of *T. brucei* from game tsetse areas is too limited to warrant any useful comparison of this species with the other two.

3. The greater adaptability of *T. rhodesiense* to tsetse has suggested reflections on the origin of this trypanosome and its affinities to *T. gambiense* and *T. brucei*, reflections that lend support to the belief that *T. rhodesiense* is in reality *T. brucei* that has overcome man's resistance.

The alternative explanation, namely, that *T. rhodesiense* is *T. gambiense* that has enjoyed a longer or shorter association with *G. morsitans* instead of with *G. palpalis*, is also considered, and

stress is laid on the need for direct experimental examination of this possibility.

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INDEX

INDEX

PAGE
INDEX OF AUTHORS i
GENERAL INDEX i
INDEX OF GENERA, SPECIES AND VARIETIES NEW TO SCIENCE vii

INDEX TO AUTHORS

PAGE
Adams, A. R. D. 1, 309
Adams, A. R. D.; and Webb, L. 471
Chatterji, R. C. 131
Corson, J. F. 481
Cushing, E. C.; and Patton, W. S. 539
Davey, T. H.; and Gordon, R. M. 27, 53, 179
Davey, T. H.; Gordon, R. M., and Hicks, E. P. 181
De Meillon, B. 83
De Meillon, B.; and Evans, A. M. 271
Duke, H. L. 99, 119, 123, 215, 355, 431, 437, 451, 569
Edwards, F. W. 357
Evans, A. M. 265
Evans, A. M.; and De Meillon, B. 271
Fairbairn, H. 185, 251
Fine, J. 180
Gibbons, E. G. 15
Gibbons, E. G.; and Loewenthal, L. J. A. 489
Gordon, R. M.; and Davey, T. H. 27, 53, 179
Gordon, R. M.; Hicks, E. P., and Davey, T. H. 181
Hawking, F.; Yorke, W., and Murgatroyd, F. 157, 347
Hicks, E. P.; Gordon, R. M., and Davey, T. H. 181
Hudson, E. H.; and Young, Agnes L. 207
Lane, Clayton 237
Lester, H. M. O. 361
Loewenthal, L. J. A.; and Gibbons, E. G. 489
Macfie, J. W. S. 79
Mer, G. 483
Murgatroyd, F.; Yorke, W., and Hawking, F. 157, 347
Nieschulz, O. 213
Patton, W. S. 135, 182, 327, 360, 397, 477, 501
Patton, W. S.; and Cushing, E. C. 539
Peaston, H. 497
Shute, P. G. 469
Stephens, J. W. W. 283
Theiler, M. 57
Wallace, J. M. 553
Webb, L.; and Adams, A. R. D. 471
Yorke, W.; Murgatroyd, F., and Hawking, F. 157, 347
Young, Agnes L.; and Hudson, E. H. . 207

GENERAL INDEX

PAGE
Acuaria spp. 5
Adams, A. R. D. A record of an investigation into the action of sera on the trypanosomes pathogenic to man .. 309
Adams, A. R. D. Report on a collection of nematodes from the Federated Malay States 1
Adams, A. R. D., and Webb, L. Two further cases of human infestation with *Bertiella studeri* (Blanchard, 1891) Stiles and Hassall, 1902, with some observations on the probable synonymy of the specimens previously recorded from man 471
America, Central and South, distribution of blackwater fever in 283
Ancylostoma brazilienze 3
  „ *caninum* 3
  „ *duodenale* 3
  „ *malayanum* 3
Animals, African game, study of the behaviour of *T. rhodesiense* in, recently isolated from man 215

PAGE

Ankylostomiasis in the Arab of the Middle Euphrates 207
Anopheles mosquitoes of Uganda 15
„ *demeilloni* sp.nov., a new name for *Anopheles transvaalensis* of authors 265
„ „ sp.nov., type form 266
„ „ Evans, type form.. 271
„ „ var. *carteri* var.nov. 276
„ „ (*transvaalensis*) and *Anopheles garnhami* in South Africa, with descriptions of new varieties of these species 271
„ *elutus* Edw., observations on
„ the development of *Plasmo-*
„ *modium malariae* Lav. in .. 483
„ *funestus* and its allies in the Transvaal 83
„ *garnhami* Edwards, type form 278
„ „ var. *walshi* var. nov. 280
„ „ and *Anopheles demeilloni* (*transvaalensis*) in South Africa, with descriptions of new varieties of these species 271
Anophelines, estimation of the density of infective, as a method of calculating the relative risk of inoculation with malaria from different species or in different localities 27
Antelope, study of the behaviour of *T. rhodesiense* in, recently isolated from man 215
Arabs of the Middle Euphrates, hookworm disease in 207
Ascaridia spp. 7
Ascaris lumbricoides 7
Bertiella studeri (Blanchard, 1891) Stiles and Hassall, 1902, in man 471
Blackwater fever, distribution of, in Central America, South America and the West Indies 283
Bunostomum phlebotomum 2
„ *trigonocephalum* 2
Capillaria spp. 7

PAGE

Chabertia ovina 3
Chatterji, R. C. On a new nematode, *Parapharyngodon maplestoni* .gen. nov., sp.nov., from a Burmese lizard 131
Chlamydonema praeputiale 6
Cochliomyia americana sp. nov., the screw-worm fly of the New World .. 539
Cooperia curticei 2
„ *pectinata* 2
„ *punctata*.................... 2
Corson, J. F. A modification of Lloyd and Johnson's method of proboscis and gland dissection in tsetse-flies.... 481
Culex aegypti L., identity of 182, 357
Culicoides, a new species of, from Palestine 79
„ *judaeae* sp. nov. 79
Cushing, E. C., and Patton, W. S. Studies on the higher Diptera of medical and veterinary importance: *Cochliomyia americana* sp. nov., the screw-worm fly of the New World .. 539
Cyclodontostomum gen. nov. 8
„ *purvisi* sp. nov. 8
Cyrnea sp. 6
Damba Island, Victoria Nyanza, the polymorphic trypanosomes of 553
Davey, T. H., and Gordon, R. M. The estimation of the density of infective anophelines as a method of calculating the relative risk of inoculation with malaria from different species or in different localities 27
Davey, T. H., and Gordon, R. M. A further note on the increase of *P. malariae* in Freetown, Sierra Leone 53
Davey, T. H., and Gordon, R. M. A note on the value of issuing free quinine to native infants............ 179
Davey, T. H.; Gordon, R. M., and Hicks, E. P. Two cases of animal rabies occurring in Sierra Leone 181
De Meillon, B. On *Anopheles funestus* and its allies in the Transvaal........ 83
De Meillon, B., and Evans, A. M. Notes on *Anopheles demeilloni* (*transvaalensis*) and *Anopheles garnhami* in South Africa, with descriptions of new varieties of these species............ 271

PAGE

Dictyocaulus filaria 4
Diptera, studies on the higher .. 135, 327, 397, 501, 539
Dirofilaria immitis 8
Drakensberg foothills, seasonal prevalence of *A. funestus* and its variety in 89
Duke, H. L. Relative susceptibility of the sexes of Glossina to infection with trypanosomes 355
Duke, H. L. Studies of the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. I : A review of existing knowledge on this subject, with some general observations 99
Duke, H. L. Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. II : On the transmitting power of different races of Glossina.......... 119
Duke, H. L. Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. III : *Glossina morsitans versus Glossina palpalis* as a transmitter of the polymorphic group of trypanosomes 123
Duke, H. L. Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. IV : On the spontaneous disappearance of flagellates from an infected Glossina 431
Duke, H. L. Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. V : On the effects of temperature.... 437
Duke, H. L. Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. VI : On the duration of the biological cycle in Glossina................. 451
Duke, H. L. Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. VII : *T. rhodesiense versus T. gambiense* : a comparison of their power to develop cyclically in Glossina...... 569
Duke, H. L. A study of the behaviour of *T. rhodesiense* recently isolated from man, in antelope and other African game animals. Part. I 215

PAGE

Edwards, F. W. The identity of *Culex aegypti* L........................ 357
Elaeophora poeli 8
Euphrates, hookworm disease in the Arab of the 207
Evans, A. M. *Anopheles demeilloni* sp. nov., a new name for *Anopheles transvaalensis* of authors 265
Evans, A. M., and De Meillon, B. Notes on *Anopheles demeilloni* (*transvaalensis*) and *Anopheles garnhami* in South Africa, with descriptions of new varieties of these species 271
Experimental infection of man with a strain of *T. rhodesiense* apparently susceptible to normal human serum *in vitro* 251
Fairbairn, H. The action of human serum *in vitro* on sixty-four recently isolated strains of *T. rhodesiense*...... 185
Fairbairn, H. Experimental infection of man with a strain of *Trypanosoma rhodesiense* apparently susceptible to normal human serum *in vitro*........ 251
Federated Malay States, collection of nematodes from 1
Fine, J. Is trypanosomiasis in the lactating rat transmitted to the sucking young ?......................... 180
Freetown, Sierra Leone, increase of *P. malariae* in 53
Gaigeria pachyscelis 2
Gibbins, E. G. The domestic *Anopheles* mosquitoes of Uganda 15
Gibbins, E. G., and Loewenthal, L. J. A. Cutaneous onchocerciasis in a *Simulium damnosum*-infested region of Uganda 489
Globocephalus urosubulatus 4
Glossina, disappearance of flagellates from an infected 431
„ duration of the biological cycle in 451
„ relation of polymorphic trypanosomes developing in the gut of, to the peritrophic membrane 347
„ relative susceptibility of the sexes of, to infection with trypanosomes 355

Glossina, transmissibility of tryparsamide-resistance by 157
  „ transmission of polymorphic trypanosomes by.......... 119
  „ *T. rhodesiense versus T. gambiense*: a comparison of their power to develop cyclically in 569
Glossina morsitans versus Glossina palpalis as transmitter of the polymorphic group of trypanosomes 123
Gnathostoma spinigerum 5
Gongylonema ingluvicola 6
Gordon, R. M., and Davey, T. H. The estimation of the density of infective anophelines as a method of calculating the relative risk of inoculation with malaria from different species or in different localities 27
Gordon, R. M., and Davey, T. H. A further note on the increase of *P. malariae* in Freetown, Sierra Leone 53
Gordon, R. M., and Davey, T. H. A note on the value of issuing free quinine to native infants........... 179
Gordon, R. M.; Hicks, E. P., and Davey, T. H. Two cases of animal rabies occurring in Sierra Leone 181
Gyalocephalus capitatus 4
Habronema spp. 6
Haemonchus contortus 1
Hawking, F.; Yorke, W., and Murgatroyd, F. The relation of polymorphic trypanosomes, developing in the gut of Glossina, to the peritrophic membrane 347
Hawking, F.; Yorke, W., and Murgatroyd, F. Studies in Chemotherapy. X.—Further observations on the transmissibility of tryparsamide-resistance by Glossina 157
Heterakis spp. 5
Hicks, E. P.; Gordon, R. M., and Davey, T. H. Two cases of animal rabies occurring in Sierra Leone 181
Hookworm disease in the Arab of the Middle Eurphrates 207
  „ larvae, infective, taxies of .. 237
Hudson, E. H., and Young, Agnes L. Hookworm disease in the Arab of the Middle Euphrates 207

Labiatofilaria gen. nov. 11
  „ *tringae* sp. nov. 11
Lane, Clayton. The taxies of infective hookworm larvae 237
Lester, H. M. O. The characteristics of some Nigerian strains of the polymorphic trypanosomes 361
Libyostrongylus douglassii.............. 2
Loewenthal, L. J. A., and Gibbins, E. G. Cutaneous onchocerciasis in a *Simulium damnosum*-infested region of Uganda 489
Macfie, J. W. S. A new species of *Culicoides* from Palestine 79
Malaria, estimation of the density of infective anophelines as a method of calculating the relative risk of inoculation with 27
  „ quartan, experimental transmission of, to man from the mosquito 483
Malaya, a new haematophagous species of *Musca* from 477
Mecistocirrus digitatus 2
Mer, G. Observations on the development of *Plasmodium malariae* Lav. in *Anopheles elutus* Edw.: experimental transmission of quartan malaria to man from the mosquito 483
Metastrongylus salmi 4
Molineus torulosus 2
Mosquitoes, *Anopheles*, of Uganda 15
Mosquitoes' eggs, a simple method of obtaining 469
Murgatroyd, F.; Yorke, W., and Hawking, F. The relation of polymorphic trypanosomes, developing in the gut of Glossina, to the peritrophic membrane 347
Murgatroyd, F.; Yorke, W., and Hawking, F. Studies in Chemotherapy. X.—Further observations on the transmissibility of tryparsamide-resistance by Glossina 157
Musca, guide to the Palaearctic species 327, 397
  „ a new haematophagous species of, from Malaya 477
  „ true blood-sucking species, as transmitters of disease 213

PAGE

Musca Linnaeus 135
„ *albina* 408
„ *autumnalis* 428
„ *convexifrons* 417
„ *crassirostris* 412
„ *domestica* 397
„ *greeni* sp. nov. 477
„ *hervei* 426
„ *larvipara* 415
„ *lucidula* 414
„ *mesopotamiensis* 420
„ *nebulo* 401
„ *sorbens* 403
„ *tempestiva* 410
„ *ventrosa* 405
„ *vicina* 399
„ *vitripennis* 407
„ *xanthomelas* 423
Muscinae 138, 501
Muscini 138, 501
Necator americanus 2
Nematode, new, from a Burmese lizard 131
Nematodes, a collection of, from the Federated Malay States 1
Nematodirus filicollis 2
Nieschulz, O. Some remarks about the rôle of true blood-sucking *Musca* species as transmitters of diseases 213
Nigerian strains of polymorphic trypanosomes, characteristics of 361
Oesophagostomum asperum 3
„ *columbianum* 3
„ *radiatum* 3
„ *venulosum* 3
Onchocerca sp. 8
Onchocerciasis, cutaneous, in a *Simulium damnosum*-infested region of Uganda 489
Ostertagia circumcincta 1
Oxyspirura spp. 6
Oxyuris equi 5
Palestine, a new species of *Culicoides* from 79
Parapharyngodon gen. nov. 131
„ *maplestoni* sp. nov. 131
Patton, W. S. The identity of *Culex aegypti* L. 182, 360
Patton, W. S. A new haematophagous species of *Musca* from Malaya 477
Patton, W. S. Studies on the higher Diptera of medical and veterinary importance: a revision of the genera of the tribe Muscini, subfamily Muscinae, based on a comparative study of the male terminalia. I.—The genus *Musca* Linnaeus 135

PAGE

Patton, W. S. Studies on the higher Diptera of medical and veterinary importance: a revision of the tribe Muscini, subfamily Muscinae, based on a comparative study of the male terminalia. II.—The genus *Stomoxys* Geoffroy (sens. lat.) 501
Patton, W. S. Studies on the higher Diptera of medical and veterinary importance: a revision of the species of the genus *Musca*, based on a comparative study of the male terminalia. II.—A practical guide to the Palaearctic species 327, 397
Patton, W. S., and Cushing, E. C. Studies on the higher Diptera of medical and veterinary importance: *Cochliomyia americana* sp. nov., the screw-worm fly of the New World 539
Peaston, H. Preliminary note on a focus of *S. mansoni* infection in Sierra Leone 497
Physocephalus sp. 6
Plasmodium malariae Lav., observations on the development of, in *Anopheles elutus* Edw. 483
„ „ comparison of life cycle of, to those of *P. vivax* and of *P. falciparum* 486
„ „ increase of, in Freetown, Sierra Leone 53
Quinine, value of issuing free, to native infants 179
Rabies, animal, in Sierra Leone 181
Rat, lactating, transmissibility of trypanosomiasis by 180
Schistosoma mansoni infection in Sierra Leone, note on a focus of 497
Screw-worm fly of the New World 539
Setaria digitata 7
„ *hornbyi* 7
„ *labiato-papillosa* 7
Shute, P. G. A simple method of obtaining eggs of mosquitoes 469

PAGE

Sierra Leone, animal rabies in 181
„ „ preliminary note on a focus of *S. mansoni* infection in 497
Simulium damnosum-infested region of Uganda, cutaneous onchocerciasis in 489
South America, distribution of blackwater fever in 283
Spirocerca sanguinolenta 6
Stephens, J. W. W. The distribution of blackwater fever in Central America, South America and the West Indies 283
Stephanurus dentatus 4
Stomoxys Geoffroy (sens. lat.) 501
„ *calcitrans*, male terminalia of 505
„ *mallochi* 535
Strongylus edentatus 4
„ *equinus* 4
„ *vulgaris* 4
Subulura sp. 5
Syngamus trachea 4
Syphacia obvelata 5
Taxies of infective hookworm larvae 237
Temperature, effects of, on the transmission of polymorphic trypanosomes by tsetse 437
Theiler, M. A yellow fever protection test in mice by intracerebral injection 57
Thelazia spp. 6
Toxocara spp. 7
Transvaal, *Anopheles funestus* and its allies in 83
Trichonema spp. 4
Trichostrongylus axei 2
„ *colubriformis* 2
„ *extenuatus* 2
„ *probolurus* 2
„ *rugatus* 2
Trichuris spp. 6
Triodontophorus brevicauda 4
„ *minor* 4
„ *serratus* 4
„ *tenuicollis* 4
T. gambiense versus T. rhodesiense: a comparison of their power to develop cyclically in Glossina 569
T. rhodesiense, action of human serum *in vitro* on sixty-four recently isolated strains of 185

PAGE

T. rhodesiense apparently susceptible to normal human serum *in vitro*, experimental infection of man with a strain of 251
„ recently isolated from man, study of the behaviour of, in antelope and other African game animals 215
„ *versus T. gambiense*: a comparison of their power to develop cyclically in Glossina 569
Trypanosomes pathogenic to man, investigation into the action of sera on 309
Trypanosomes, polymorphic, characteristics of some Nigerian strains of 361
„ polymorphic, developing in the gut of Glossina, relation of, to the peritrophic membrane 347
„ polymorphic, of Damba Island, Victoria Nyanza 553
„ polymorphic, transmission of, by tsetse 99, 119, 123, 431, 437, 451, 569
„ relative susceptibility of the sexes of Glossina to infection with 355
Trypanosomiasis, transmissibility of, by the lactating rat 180
Tryparsamide-resistance, transmissibility of, by Glossina 157
Tsetse, transmission of polymorphic trypanosomes by 99, 119, 123, 431, 437, 451, 569
„ -flies, proboscis and gland dissection in 481
Uganda, cutaneous onchocerciasis in a *Simulium damnosum*-infested region of 489
„ domestic *Anopheles* mosquitoes of 15
Wallace, J. M. The polymorphic trypanosomes of Damba Island, Victoria Nyanza. III.—Their reactions in laboratory animals 553

PAGE

Webb, L., and Adams, A. R. D. Two further cases of human infestation with *Bertiella studeri* (Blanchard, 1891) Stiles and Hassall, 1902, with some observations on the probable synonymy of the specimens previously recorded from man 471
West Indies, distribution of blackwater fever in 283
Yellow fever protection test in mice by intracerebral injection 57
Yorke, W.; Murgatroyd, F., and Hawking, F. The relation of polymorphic trypanosomes, developing in the gut of Glossina, to the peritrophic membrane 347
Yorke, W.; Murgatroyd, F., and Hawking, F. Studies in Chemotherapy. X.—Further observations on the transmissibility of tryparsamide-resistance by Glossina 157
Young, Agnes L., and Hudson, E. H. Hookworm disease in the Arab of the Middle Euphrates 207

INDEX OF GENERA, SPECIES AND VARIETIES NEW TO SCIENCE

PAGE

Anopheles demeilloni sp. nov., type form 266
Anopheles demeilloni var. *carteri* var. nov. 276
Anopheles garnhami var. *walshi* var. nov. 280
Cochliomyia americana sp. nov. 539
Culicoides judaeae sp. nov. 79
Cyclodontostomum gen. nov. 8
Cyclodontostomum purvisi sp. nov. 8
Labiatofilaria gen. nov. 11
Labiatofilaria tringae sp. nov. 11
Musca greeni sp. nov. 477
Parapharyngodon gen. nov. 131
Parapharyngodon maplestoni sp. nov..... 131

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